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Intake of Live Microorganisms in Adults and Its Impact on Microbiota and Health Parameters

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Abstract

The intake of live microorganisms (LMOs) may contribute to modulating gut microbial ecology with an impact on health. In this study, we examined the intake of LMOs in adults and its association with gut microbiota composition, intestinal short-chain fatty acids (SCFAs), and health-related biochemical parameters. A total of 151 adults were analyzed across three age groups (18–50, 51–65, and 66–95 years). Dietary intake was assessed using a food frequency questionnaire (FFQ), and LMOs consumption was estimated with a previously developed database. The levels of some relevant intestinal microbial groups were measured using Quantitative Polymerase Chain Reaction (qPCR), SCFAs were determined using gas chromatography, and biochemical markers were assessed through standardized laboratory methods. LMOs intake was significantly higher in the two older age groups compared with younger adults, with yogurt identified as the primary dietary source of LMOs across all ages. In the middle-aged group, bacterial LMOs intake independently predicted higher abundances of *Akkermansia* and *Bacteroides*-related taxa, while fungal LMOs were positively associated with butyric acid levels. In the older age group, bacterial LMOs intake was directly associated with branched-chain fatty acids (BCFAs) while fungal LMOs intake was directly associated with circulating cholesterol and inversely with malondialdehyde (MDA). Overall, the findings suggest that LMOs consumption, mainly bacteria intake from fermented dairy products, increases with age and is linked to age-specific changes in gut microbiota, microbial metabolites, and biochemical health parameters.

Keywords: live microorganisms; microbiota; health; elderly; fermented foods

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1. Introduction

Dietary live microorganisms (LMOs) have emerged as a relevant, yet still insufficiently characterized, component of the human diet [1]. LMOs are defined as bacteria and fungi that remain viable at the time of consumption and are naturally present in foods or introduced during food processing, without necessarily being administered as defined strains [2]. In contrast to probiotics, which are evaluated at specific doses and for strain-specific health effects, LMOs comprise a broad and diverse group of microorganisms consumed habitually through conventional foods, representing a continuous form of microbial exposure embedded within the habitual diet.

According to Marco et al. [2], fermented foods, including yogurt, fermented milks, cheeses, and other cultured non-dairy products, constitute the main dietary sources of LMOs in most Western populations. These foods often provide microbial loads ranging from 10^6 to 10^9 colony-forming units (CFU) per serving, depending on the food matrix, fermentation process, and storage conditions. Current evidence has shown that the intake of 100 g of foods with a medium or high microbial load may be associated with the improvement in cardiometabolic risk factors, including cholesterol and lipoprotein levels [3]. In this context, a microbial daily dose of $\geq 2 \times 10^9$ CFU has been proposed to be associated with non-adverse health outcomes, based on the available evidence from specific foods or probiotic products [4], which is comparable with the intake estimated within plant-based dietary patterns (1×10^9 CFU/day) [5]. Subsequent work by Marco et al. [6] emphasized that fermented foods should be regarded as complex matrices delivering not only LMOs but also fermentation-derived metabolites, which together may interact with the resident gut microbiota. Importantly, these authors highlighted that, despite the global and long-standing consumption of foods containing live microbes, the habitual intake of LMOs has rarely been quantified at the population level.

Until recently, research addressing dietary LMOs had largely focused on isolated food groups, most notably fermented dairy products, or on intervention studies conducted in controlled settings. Thus, limited evidence is available regarding total dietary LMOs exposure derived from multiple food sources under free-living conditions. In addition, the intake and potential role of fungal LMOs have received very limited attention, despite their substantial contribution to total dietary intake of microbes [7]. In recent years, the assessment and health consequences of the dietary intake of LMOs have attracted attention, and reports have been published which focused on adults [3,8–12] and children [7]. These studies, mostly based in the qualitative categorization of foods on the basis of LMOs levels, have underlined the association between LMOs intake and health-related biochemical parameters [3] and clinically relevant aspects such as cognitive function [11] or cardiovascular disease and mortality [10]. Associations between LMOs intake and gut microbiota have also been reported [7], although the available information is still limited. Moreover, the absence of population-based data on dietary LMOs is especially relevant in elderly individuals. Older adults often exhibit distinct dietary patterns, characterized by a reduced total energy intake, lower dietary diversity, and altered consumption of fermented foods [13]. In parallel, aging has been consistently associated with pronounced changes in gut microbiota composition and altered biochemical profiles [14,15]. Reductions in saccharolytic and butyrate-producing bacteria, such as *Bifidobacterium*, *Faecalibacterium prausnitzii*, and members of *Clostridium* cluster XIVa, have been reported, together with increased abundances of lactic acid bacteria and mucin-degrading microorganisms such as *Akkermansia muciniphila* [16]. These age-associated microbial patterns have been linked to reduced fermentative capacity and lower production of short-chain fatty acids (SCFAs). Despite the recognized susceptibility of the gut microbiota to dietary modulation in later life [17], the habitual intake of dietary LMOs in elderly populations has been positively associated with improved cognitive function and reduced frailty [11,12,18]. However, existing studies have largely relied on broad categorizations of foods according to microbial load, rather than systematically quantifying habitual LMOs intake based on CFU/day from individual foods and identifying the main dietary sources. Consequently, the extent to which exposure to LMOs through foods may be associated with gut microbiota composition, microbial metabolite production, and health-related biomarkers in older adults remains largely unknown. This gap was highlighted by Marco et al. [8], calling for observational studies that quantify LMOs intake in specific populations. Based on this background, it was hypothesized that the habitual intake of dietary LMOs is substantial yet heterogeneous in

elderly individuals and that higher intake, particularly from fermented foods, is associated with distinct fecal microbial profiles and altered SCFAs patterns in older adults.

Therefore, the primary objective of the present study was to quantitatively characterize the habitual intake of dietary LMOs and their main food sources in an adult population, with particular emphasis on elderly individuals, a population for which such data are currently lacking. Secondary objectives were to explore the associations between dietary LMOs intake and relevant fecal microbial groups, microbial metabolites, and selected anthropometric and biochemical parameters, and to assess whether these relationships differ according to age. By addressing these objectives, this work aimed to fill a critical gap in the literature regarding the intake of LMOs and their potential role in modulating the gut microbiota in later life.

2. Materials and Methods

2.1. Participants and Study Design

The sample in this observational study comprised 151 healthy adults aged 19 to 95 years, recruited in the Asturias region (north of Spain). The inclusion criteria were no diagnosis with cancer, Parkinson's disease, irritable bowel disease or autoimmune diseases and not having consumed antibiotics or probiotics during the month before recruitment. Ethical approval was granted by the Regional Ethics Committee for Clinical Research of the Principality of Asturias, in accordance with the principles of the Declaration of Helsinki (1964), last revised in 2013. Written informed consent was obtained from all participants, and all study procedures were conducted in compliance with updated and approved institutional guidelines and regulations.

2.2. General and Anthropometric Characteristics of the Study Sample

Personal interviews were conducted, and general characteristics, including gender and age (n = 151; male/female and years), were recorded. Lifestyle variables were also documented, including sleeping duration (n = 74; hours/day), sedentarism (n = 114; $\leq$29.99 min walking/day), and smoking status (n = 70; current, former or never smoker). In addition, weight (kg) and height (m) were measured (n = 146) using standardized methods, and the Body Mass Index (BMI) was calculated (weight (kg)/height $(m)^2$). BMI categories were created following the criteria of the Spanish Society for the Study of Obesity (SEEDO): normal weight ($\leq$24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obese ($\geq$30.0 kg/m^2) [19].

2.3. Nutritional Assessment

Dietary intake was assessed during personal interviews (n = 151) using a semiquantitative Food Frequency Questionnaire (FFQ) adapted for the Spanish population and previously validated by our research group, as described elsewhere [20]. The FFQ included 160 items, and food consumption was classified into food groups according to the Centre for Higher Education in Nutrition and Dietetics (CESNID) [21]. Energy and macronutrient intakes were estimated using the CESNID food composition tables [21]. The consumption of different subtypes of fiber (soluble and insoluble fiber, insoluble cellulose, Klason lignin, soluble and insoluble hemicellulose and soluble and insoluble pectin) was estimated using Marlett and Cheung tables [22], and for the content of the main classes of (poly)phenol we used the Phenol-Explorer database [23]. Foods were classified according to their content in LMOs following Marco et al. criteria: low (<10^4 CFU/g), medium (10^4–10^7 CFU/g), and high (>10^7 CFU/g) [8], and the total intake of fermented foods was estimated [21]. Finally, the intake of total LMOs was calculated using a database previously developed and validated [7].

2.4. Fecal Microbiota and Metabolites Analyses

The fecal microbiota was analyzed following the previously described methods (n = 139) [16,20]. Briefly, fecal samples were obtained immediately after defecation in sterile containers, frozen at −20 °C, and subsequently transported to the laboratory for further analysis. For DNA extraction, 1 g of each sample was weighed, diluted 1:10 in sterile PBS solution, and homogenized using a LabBlender 400 stomacher (Seward Medical, London, UK) at full speed for 3 min. Then, samples were centrifuged at 10,000× *g* for 30 min at 4 °C, and the supernatant and bacterial pellet were separated. DNA was extracted from the bacterial pellet following the Q protocol for DNA extraction from fecal samples defined by the International Human Microbiome Standards Consortium [24]. Then, fecal microbiota groups (*Akkermansia*, *Bacteroides* group, *Bifidobacterium*, *Clostridium* cluster XIVa, *Lactobacillus* group and *Faecalibacterium*) were quantified using Quantitative Polymerase Chain Reaction (qPCR), with the primers and conditions previously detailed [20]. A 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and SYBR Green chemistry (Applied Biosystems, Foster City, CA, USA) were used for the qPCR reactions. Standard curves made with pure cultures of the correspondent bacterial strains were included to calculate the bacterial levels (log of the number of cells per gram of feces).

The quantification of SCFAs was performed using gas chromatography coupled to a flame detector (Agilent Technologies. Inc, Santa Clara, CA, USA) on previously obtained and filtered fecal supernatants, following the established analytical procedures described elsewhere [25].

2.5. Blood Biochemical Analyses

Blood biochemical analyses were performed (n = 132), as explained elsewhere [20]. Briefly, fasting blood samples were drawn using venipuncture, collected in separate tubes for serum and plasma, kept on ice and centrifuged (1000× *g*, 15 min) within 2–4 h after collection. Aliquots of serum and plasma were stored at −20 °C until further analyses. Plasma glucose, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides were determined via standard methods using an automated biochemical autoanalyzer. Serum levels of C-reactive protein (CRP) and leptin were determined using ELISA kits (CRP Human Instant ELISA kit, Ebioscience, San Diego, CA, USA, and Human Leptin ELISA Development Kit, 900-K90 PeproTech Inc., Rocky Hill, NJ, USA, respectively). Malondialdehyde (MDA) was determined by means of a colorimetric assay of lipid peroxidation (Byoxytech LPO-586, Oxis International S.A., Paris, France).

2.6. Statistical Analysis

The results were analyzed using IBM SPSS 25.0 (IBM SPSS, Inc., Chicago, IL, USA) and Rstudio (v. 2024.09.0+375). The goodness of fit to the normal distribution was checked by means of the Kolmogorov–Smirnov test. Overall, the categorical variables were summarized as numbers and percentages and continuous ones as the mean and standard deviation (SD) or median and 25th and 75th percentiles (P_{25}–P_{75}). The Fisher test and Mann–Whitney U test were used to compare categorical and continuous values, respectively. A Benjamini–Hochberg correction was applied to adjust for multiple comparisons using the p.adjust function from the stats R package (v.4.4.2; R Core Team, 2025). Spearman correlation analyses were conducted to study the relationship between the intake of LMOs (total, bacterial and fungal types) and the fecal microbiota, SCFAs, and anthropometric and biochemical parameters. To further analyze this relationship, stepwise regression analyses were performed, using those significantly correlated with LMO intake (total, bacteria, and/or fungi) as dependent variables and adjusting for gender, BMI, alcohol consumption,

daily energy intake, and the intake of fibers and polyphenols. For graphical representations, ggplot function from ggplot2 [26], and pheatmap function from pheatmap [27] were used.

3. Results

The general, anthropometric, and biochemical characteristics of the participants are summarized in Tables S1 and S2 shows the daily energy intake and consumption of the main food groups.

The consumption of foods with a medium content of LMOs or with a medium or high content of LMOs was lower in the elderly compared with the middle-age group (25 vs. 152 and 109 vs. 272 g/day, respectively) (Table 1). In addition, the middle-age group presented the highest intake of fermented foods (300 g/day), followed by the younger group (150 g/day) and the older group (73 g/day).

Table 1. Daily intake of food with low (<10^4 CFU/g), medium (10^4–10^7 CFU/g), and high (>10^7 CFU/g) contents of live microorganisms (LMOs), and fermented foods (g/day).

Food (g/day)	18–50 Years n = 51	51–65 Years n = 55	66–95 Years n = 45
Low LMOs	1350.96 (1149.79–1659.87)[a]	1444.49 (1221.79–1781.46)[a]	1366.79 (1129.68–1580.28)[a]
Medium LMOs	132.93 (57.00–195.44) [a]	151.50 (99.32–265.40)[a]	24.50 (0.00–49.86)[b]
High LMOs	62.50 (25.15–136.43)[a]	125.00 (45.00–187.50)[a]	71.25 (17.50–156.25)[a]
Medium or High LMOs	207.17 (123.57–334.37)[a]	272.25 (152.89–469.27)[b]	108.87 (46.74–232.34)[c]
Fermented	150.00 (46.43–241.79)[a]	300.00 (133.93–375.00)[b]	73.25 (22.82–196.25)[a]

The data are presented as the median (P_{25}–P_{75}). The values in the same row with different letters represent statistically significant differences between age groups according to the Mann–Whitney U test (p-value < 0.05, adjusted for multiple comparisons analyses using the Benjamini–Hochberg method). LMOs: live microorganisms.

The daily intakes of LMOs, bacteria, and fungi are shown in Figure 1. The median LMOs intake across age groups ranged from 9.6 to 10.3 log CFU/day (Figure 1a). The bacteria intake was higher in the 51–65 years and 66–95 years age groups compared with the youngest groups (10.3 and 10.1 vs. 9.6 log CFU/day) (Figure 1b), whereas the intake of fungi was lower in the older group (7.7 and 7.8 vs. 7.4 log CFU/day) (Figure 1c).

Dairy products such as yogurt, mainly whole plain, and Manchego-type semi-matured cheese represented the predominant contributors to the total LMOs and bacterial intake in the 51–65 and 66–95 years age groups (Figure 2a,b). The youngest group presented a more heterogeneous distribution of dietary total LMOs and bacterial sources since cooked ham, Manchego-type matured cheese, "chorizo", and lettuce were additional contributors. In the case of the total dietary intake of fungi, lettuce in the 66–95 year-old group and tomato in the 51–65 and 18–50 year-old groups were the largest contributors (Figure 2c).

The daily intake of dietary sources of LMOs is shown in Table 2. Yogurt consumption was significantly higher in both older groups compared with the youngest adults (98–99 vs. 21 g/day). The 18–50 and 51–65 year-old groups presented higher consumption of matured Manchego cheese and semi-matured Manchego cheese (4 and 15 g/day, respectively). The intake of fermented meats such as cooked ham and cured sausage with paprika, "chorizo", differed across age groups, with the youngest adults consuming more cooked ham (18 g/day) and the oldest consuming more cured sausage with paprika, "chorizo" (6 g/day). The contribution of raw vegetables to LMOs intake also varied, with the oldest group reporting the lowest lettuce and tomato intake (23 and 4 g/day).

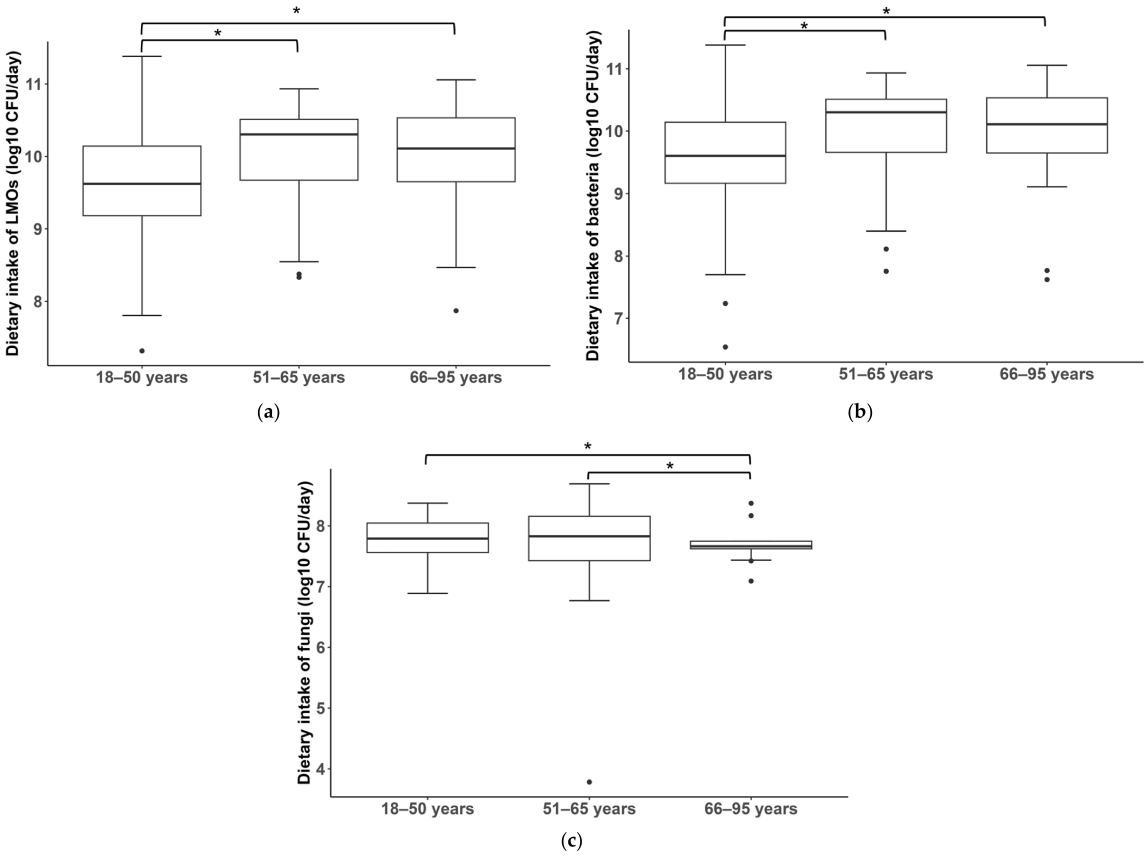

Figure 1. Intake of (**a**) live microorganisms (LMOs), (**b**) bacteria, and (**c**) fungi ($\log_{10}$ CFU/day) according to age group. The lines within the boxes represent the median, and the bounds of boxes represent the first and third quartiles (25th and 75th percentiles, respectively). The whiskers denote the lowest and highest values within 1.5 times the interquartile range (IQR) from the first and third quartiles and dots represent individual observations outside the whiskers of the boxplot (outliers). * p-value < 0.05 according to the Mann–Whitney U test and adjusted for multiple comparisons analyses using the Benjamini–Hochberg method.

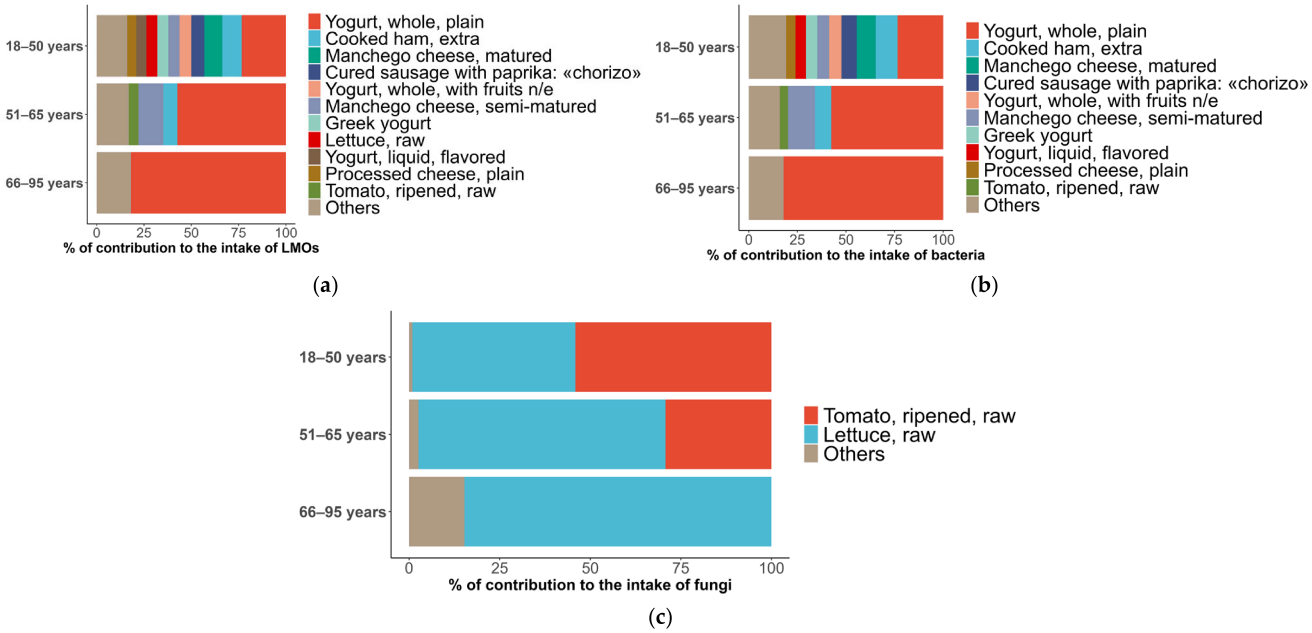

Figure 2. Dietary sources of (**a**) live microorganisms (LMOs), (**b**) bacteria, and (**c**) fungi according to age groups. The data were calculated only for consumers of LMOs.

Table 2. Daily intake of the main dietary sources of live microorganisms (LMOs) according to age groups.

g/day	18–50 Years (n = 51)	51–65 Years (n = 55)	66–95 Years (n = 45)
Yogurt, whole, plain	20.57 ± 40.97[a]	98.71 ± 107.31[b]	98.33 ± 113.19[b]
Yogurt, whole, with fruits n/e	3.15 ± 11.27[a]	1.30 ± 6.96[a]	0.00 ±0.00[a]
Greek yogurt	5.78 ± 25.12[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Yogurt, liquid, flavored	8.12 ± 39.20[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Manchego cheese, matured	3.82 ± 8.71[a]	1.32 ± 6.72[a,b]	0.00 ± 0.00[b]
Manchego cheese, semi-matured	2.08 ± 6.01[a]	15.49 ± 29.69[b]	3.01 ± 7.73[b]
Processed cheese, plain	0.88 ± 3.82[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]
Cooked ham, extra	17.92 ± 24.60[a]	7.46 ± 14.66[b]	11.75 ± 14.88[a]
Cured sausage with paprika «chorizo»	2.63 ± 4.01[a]	2.33 ± 6.47[a]	5.96 ± 6.75[b]
Lettuce, raw	29.44 ± 25.91[a,b]	62.06 ± 76.23[a]	22.85 ± 33.32[b]
Tomato, ripened, raw	46.03 ± 37.13[a]	32.59 ± 73.34[b]	4.41 ± 6.68[c]

The data are presented as means ± standard deviations (SDs). The values in the same row with different letters represent statistically significant differences between age groups according to the Mann–Whitney U test (p-value < 0.05, adjusted for multiple comparisons analyses using the Benjamini–Hochberg method).

Regarding the levels of the intestinal microbial groups analyzed, some differences were observed between the age groups (Table 3). Significant age-related differences were also observed in SCFAs. Acetic, propionic and butyric acids showed a clear decreasing gradient across age groups, with the highest concentrations in the youngest participants and the lowest in the oldest group. In contrast, branched-chain fatty acids (BCFAs), isobutyric acid, isovaleric acid and caproic acid did not differ significantly between age groups.

Table 3. Absolute abundance of fecal microbiota and levels of short-chain fatty acids (SCFAs) according to age group.

Fecal	18–50 Years n = 44	51–65 Years n = 53	66–95 Years n = 42
Microorganism (log_{10} cells/g)			
Akkermansia	5.33 (4.00–7.50)[a]	5.38 (4.33–7.56)[a]	7.43 (5.86–8.73)[b]
Bacteroides-Prevotella-Porphyromonas	8.62 (7.37–9.74)[a,b]	9.23 (8.53–9.76)[a]	8.88 (8.35–9.21)[b]
Bifidobacterium	8.57 (8.28–8.89)[a]	8.22 (7.81–8.69)[a]	7.58 (6.99–8.43)[b]
Clostridium cluster XIVa	8.77 (8.13–9.16)[a]	8.36 (7.56–8.97)[a]	6.46 (4.63–7.52)[b]
Lactobacillus group	5.98 (5.45–6.90)[a]	5.88 (5.30–6.77)[a]	7.27 (5.63–7.98)[b]
Faecalibacterium prausnitzii	8.11 (7.29–8.71)[a]	7.47 (6.86–8.1)[b]	6.52 (5.88–7.07)[c]
SCFAs (mM)	n = 43	n = 52	n = 37
Acetic acid	52.08 (40.52–63.04)[a]	32.27 (24.16–38.40)[b]	17.75 (11.76–29.98)[c]
Propionic acid	15.49 (9.34–22.08)[a]	12.395 (8.1–16.82)[b]	7.20 (3.57–12.76)[c]
Butyric acid	10.00 (7.05–13.67)[a]	9.56 (5.04–13.51)[a,b]	5.84 (3.45–11.07)[b]
Isobutyric acid	1.42 (0.97–1.78)[a]	1.44 (0.93–2.14)[a]	1.45 (1.09–2.16)[a]
Isovaleric acid	2.01 (1.29–2.52)[a]	2.16 (1.14–3.28)[a]	1.87 (1.40–3.15)[a]
Valeric acid	1.88 (1.34–2.56)[a]	1.74 (1.13–2.61)[a,b]	0.93 (0.56–1.78)[b]
Caproic acid	0.40 (0.22–0.88)[a]	0.00 (0.00–0.18)[b]	0.00 (0.00–0.00)[a,b]
APB	81.42 (55.16–96.18)[a]	53.68 (41.06–67.08)[b]	30.85 (19.90–52.29)[c]
BCFAs	3.33 (2.40–4.36)[a]	3.44 (2.20–5.44)[a]	3.36 (2.53–5.23)[a]

The data are presented as the median (P_{25}–P_{75}). The values in the same row with different letters represent statistically significant differences between age groups according to the Mann–Whitney U test (p-value < 0.05, adjusted for multiple comparisons analyses using the Benjamini–Hochberg method). APB: sum of acetic, propionic and butyric acids; BCFAs: branched-chain fatty acids; SCFAs: short-chain fatty acids.

To explore age-specific associations between the intake of LMOs and gut microbiota, Spearman correlation heatmaps were generated (Figures 3 and 4). In the middle age group, the intake of total LMOs and bacteria showed positive correlations with intestinal levels of

Akkermansia and *Bacteroides–Prevotella–Porphyromonas*, while in the 66–95 year-old group, negative correlations were found with *Bifidobacterium* and positive correlations with isovaleric acid, isobutyric acid and BCFAs (Figure 3). The intake of fungi was directly associated with the fecal levels of butyric acid in the middle age group and with *Faecalibacterium prausnitzii* in the oldest group. Associations between the intake of LMOs, bacteria, or fungi and with circulating levels of health parameters also differed by age (Figure 4). In the middle age group, fungi intake was inversely associated with fasting glucose levels whereas in the older age group, fungi intake was inversely associated with the oxidative stress marker MDA and directly associated with LDL and total cholesterol.

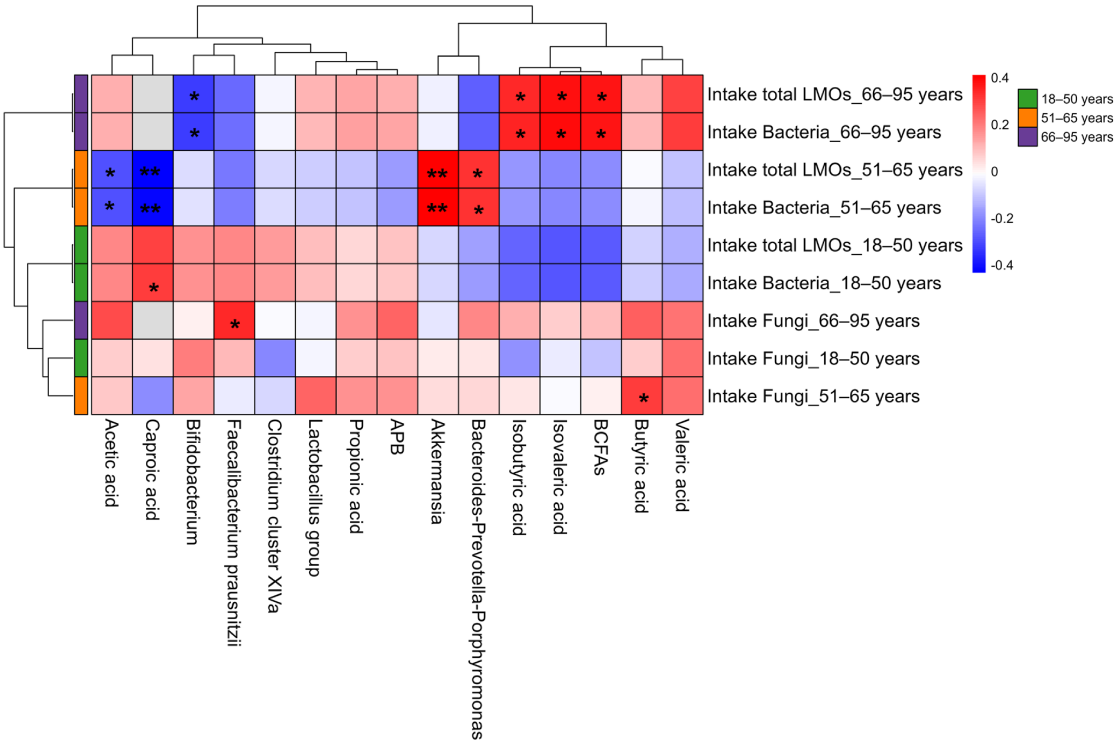

Figure 3. Heatmap defined by Spearman correlations between the intake of total live microorganisms (LMOs), bacteria and fungi (CFU/day) and fecal microbiota (log_{10} cells/g) and metabolites (SCFAs, mM) clustered by age group. The blue and red colors denote negative and positive associations, respectively. The intensity of the color is proportional to the degree of association between variables. * *p*-value < 0.05; ** *p*-value < 0.01. LMOs, live microorganisms; SCFAs: short-chain fatty acids; APB: sum of acetic, propionic and butyric acids; BCFAs: branched-chain fatty acids.

Variables showing significant Spearman associations with the consumption of LMOs (total, bacteria and/or fungi) were introduced into stepwise regression models to identify independent dietary predictors of fecal microbiota, SCFAs, and health-related parameters (Table 4).

In the middle age group, the bacterial intake independently predicted higher abundances of *Akkermansia* (R^2 = 0.124, *p*-value = 0.006) and *Bacteroides–Prevotella–Porphyromonas* (R^2 = 0.082, *p*-value = 0.021), while the fungal intake was positively associated with butyric acid (R^2 = 0.096; *p*-value = 0.014), an effect that strengthened after adjustment for polyphenol intake (R^2 = 0.172). In the oldest group, lignan intake emerged as the strongest predictor of isobutyric acid (R^2 = 0.390, *p*-value < 0.001), and this association remained significant in multivariable models that included bacterial intake (R^2 = 0.508). Bacteria consumption was the main predictor of isovaleric acid and BCFAs (R^2 = 0.351 and R^2 = 0.354, respectively), with increasing explanatory power after adjustment for insoluble cellulose, polyphenols, and lignans (R^2 = 0.509 and R^2 = 0.587, respectively). In the 66–95-year-old group, fungi in-

take was the strongest predictor of MDA (R^2 = 0.245, *p*-value < 0.001), showing an increased explanatory power after adjustment for polyphenol intake (R^2 = 0.317).

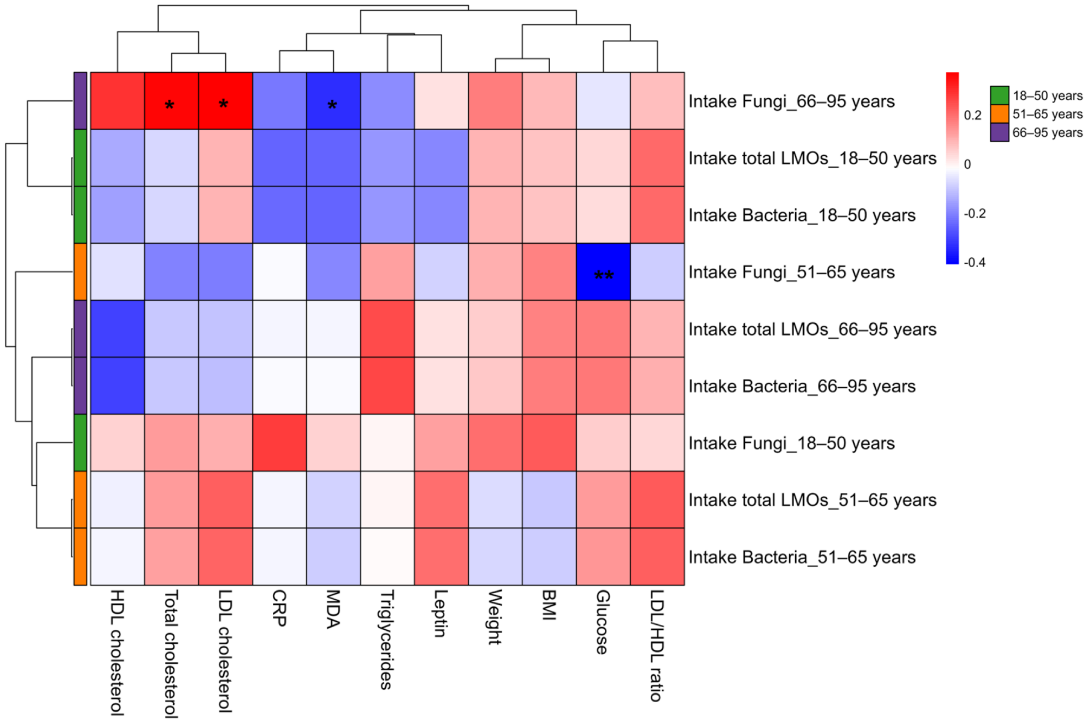

Figure 4. Heatmap defined by Spearman correlations between the intake of total live microorganisms (LMOs), bacteria and fungi (CFU/day) and health parameters (weight (kg), body mass index (BMI, kg/m^2), glucose (mg/dL), triglycerides (mg/dL), total cholesterol (mg/dL), high-density lipoprotein (HDL) cholesterol (mg/dL), low-density lipoprotein (LDL) cholesterol (mg/dL), LDL/HDL ratio, leptin (ng/mL), C-reactive protein (CRP, mg/L) and malondialdehyde (MDA, μM)), clustered by age group. The blue and red colors denote negative and positive associations, respectively. The intensity of the color is proportional to the degree of association between variables. * *p*-value < 0.05; ** *p*-value < 0.01. LMOs: live microorganisms; BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; CRP: C-reactive protein; MDA: malondialdehyde.

Table 4. Results obtained from stepwise regression analyses identifying food components that modify the fecal microbiota and metabolites (SCFAs) and health parameters.

Age Group	Dependent Variable		Independent Variable	R^2	β	*p*-Value
51–65 years	*Akkermansia*	Model 1	Intake of bacteria	0.124	0.376	0.006
	Bacteroides-Prevotella-Porphyromonas	Model 1	Intake of bacteria	0.082	0.316	0.021
	Butyric acid	Model 1	Intake of fungi	0.096	0.338	0.014
		Model 2	Intake of fungi	0.172	0.376	0.005
			Intake of total polyphenols		−0.302	0.023
66–95 years	Isobutyric acid	Model 1	Intake of lignans	0.390	0.639	<0.001
		Model 2	Intake of lignans	0.508	0.456	0.002
			Intake of bacteria		0.404	0.006

Table 4. *Cont.*

Age Group	Dependent Variable		Independent Variable	R^2	β	*p*-Value
	Isovaleric acid	Model 1	Intake of bacteria	0.351	0.609	<0.001
		Model 2	Intake of bacteria	0.434	0.559	<0.001
			Intake of insoluble cellulose		0.317	0.023
		Model 3	Intake of bacteria	0.509	0.525	<0.001
			Intake of insoluble cellulose		0.359	0.007
			Gender		−0.296	0.023
	BCFAs	Model 1	Intake of bacteria	0.354	0.611	<0.001
		Model 2	Intake of bacteria	0.471	0.429	0.005
			Intake of lignans		0.403	0.008
		Model 3	Intake of bacteria	0.523	0.366	0.013
			Intake of lignans		0.962	0.003
			Intake of total polyphenols		−0.590	0.045
		Model 4	Intake of bacteria	0.587	0.383	0.006
			Intake of lignans		0.969	0.002
			Intake of total polyphenols		−0.821	0.006
			Intake of insoluble cellulose		0.345	0.024
	MDA	Model 1	Intake of fungi	0.245	−0.514	0.001
		Model 2	Intake of fungi	0.317	−0.422	0.004
			Intake of total polyphenols		−0.311	0.031

Stepwise regression analysis was adjusted for gender (male as reference category), body mass index (BMI), alcohol consumption, daily energy intake, and intake of each fiber subtype and each polyphenol category. Only independent variables with *p*-value < 0.05 are shown. R^2: coefficient of multiple determination; β: standardized regression coefficient; BCFAs: branched-chain fatty acids; MDA: malondialdehyde.

4. Discussion

The present study provides an integrated assessment of age-related differences in lifestyle factors, dietary intake of LMOs, gut microbiota composition, microbial metabolite production, and selected health biomarkers in adults spanning early adulthood to advanced age. The results indicate that aging is associated with marked changes, in not only gut microbial ecology but also in the food sources and physiological correlations with dietary LMOs.

A major finding of this study was the clear age dependency of the total LMOs intake and its dietary sources. Yogurt consumption was significantly higher in middle-aged and older adults, confirming fermented dairy products as the predominant contributors to total and bacterial LMOs exposure in these age groups. This observation is consistent with previous observational and intervention studies showing that yogurt is the most frequently consumed fermented food in adult and elderly populations [28,29]. In contrast, younger adults exhibited a lower intake of LMOs and a more heterogeneous distribution of dietary LMOs sources. Although the literature quantifying LMOs intake from non-dairy foods such as cooked ham remains extremely limited, the level of consumption of these foods containing low LMOs in our sample population (1350–1450 g/day) is similar to the values reported for US (Med: 106 g/day) and Australian (Med: 254 g/day) adults [8,9]. Similarly, younger and middle-aged adults exhibited the highest fungal LMOs intake levels, likely reflecting the greater consumption of raw vegetables, particularly lettuce (29 and 62 g/day, respectively) and tomato (46 and 33 g/day, respectively). Most available studies have focused almost exclusively on fermented dairy products, and therefore direct comparisons regarding the contribution of fermented meats or raw vegetables to gut microbiota modulation cannot be made [30]. The present results thus provide novel population-level data in an area that remains largely unexplored.

Pronounced age-related differences were also observed in fecal microbiota. In agreement with previous observations, older adults exhibited significantly higher abundances of *Akkermansia* and the *Lactobacillus* group, whereas *Bifidobacterium*, *Faecalibacterium prausnitzii*, and *Clostridium* cluster XIVa were significantly reduced [16]. The enrichment of *Akkermansia* has been consistently reported [31,32] and linked to improved gut barrier function and metabolic health [32], and the higher abundance of *Lactobacillus* observed in older adults in the present study may reflect increased consumption of fermented dairy products [28]. Interestingly, age-specific associations were observed between dietary LMOs and gut microbiota composition. In the middle age group, bacterial LMOs intake independently predicted higher abundances of *Akkermansia* and the *Bacteroides–Prevotella–Porphyromonas* group, consistent with previous findings showing increased *Akkermansia* levels in regular yogurt consumers [28]. *A. muciniphila* is known to stimulate mucin turnover, reinforce epithelial tight-junction pathways, and modulate host inflammatory signaling, thereby strengthening mucosal barrier function [33]. Moreover, *A. muciniphila* has been consistently associated with improved metabolic health, including enhanced insulin sensitivity, reduced adipose inflammation, and healthier aging trajectories [34]. Nevertheless, most published studies have assessed fermented dairy intake rather than total dietary LMOs exposure, and therefore comparisons should be interpreted with caution.

The total fecal SCFAs concentrations were also in agreement with previous reports [16,35]. In contrast, BCFAs did not differ significantly between age groups. Similar observations have been reported previously and suggest that protein fermentation may be relatively preserved during aging, even in the context of reduced carbohydrate fermentation [36]. However, human data on age-related changes in BCFAs remain scarce, limiting firm conclusions. In the oldest adult group, the dietary intake of bacterial LMOs was the main predictor of BCFAs. To the authors' knowledge, no previous studies have directly examined the relationship between dietary LMOs intake and protein fermentation markers in older populations, highlighting a gap in current microbiome–nutrition research. BCFAs have been proposed to exert context-dependent effects but their physiological significance remains incompletely understood. The association with bacterial LMOs intake, mainly related to fermented milks intake in this population group, may be suggestive that the microorganisms, or metabolites, from these fermented foods modulate nitrogen within the gut ecosystem thereby promoting BCFA production. However, on the basis of our data the role of age-specific dietary patterns, such as lower fiber intake or the different protein distribution, favoring BCFA formation independently of LMOs cannot be disregarded.

Interestingly, fungal LMOs intake emerged as an independent predictor of fecal butyrate in middle-aged adults and was inversely associated with circulating MDA concentrations in older adults. Many LMOs may produce metabolites such as lactate and acetate, which serve as substrates for butyrate production through cross-feeding mechanisms by taxa such as *F. prausnitzii*, *Eubacterium rectale* and *Roseburia* spp. [37]. Additionally, LMOs can modulate the luminal pH and mucosal nutrient gradients through the production of organic acids, creating ecological conditions that selectively favor butyrate-producing Firmicutes [38]. With regard to the association with MDA, while raw vegetables and fermented foods have been linked to reduced oxidative stress, the specific contribution of LMOs has rarely been investigated, and no human studies specifically addressing this association could be identified [29,39]. In this regard, fermentation not only provides microorganisms but also enhances the properties of foods through the generation of bioactive compounds with antioxidant activity [2]. Moreover, fermented foods containing LMOs can modulate gut microbial composition and function, leading to the production of metabolites that influence inflammatory and redox pathways [40]. It is important to underline that although several mechanisms may explain the observed associations, our

findings about fungal LMOs or the relationship between LMOs and blood lipids may be influenced by dietary variables that may act as confounders and, therefore, should be considered exploratory.

In conclusion, the present study demonstrates that aging is associated with profound changes in dietary LMOs intake patterns, gut microbiota composition, and microbial metabolites. The associations between LMOs, gut microbiota, and health markers were found to be strongly age-dependent. While fermented dairy products were the main contributors to LMOs intake in older adults, non-dairy sources contributed substantially in younger individuals, despite the lack of supporting literature addressing their biological relevance. In the context of this study, it is also important to consider that foods represent complex food matrices, including yogurt and other fermented products, and contain numerous bioactive constituents beyond LMOs, many of which may be independently associated with antioxidant and anti-inflammatory effects [2]. Although several physiological changes are known to occur with aging, LMOs intake may represent a previously unexplored factor in this process. In the older age group, yogurt represented the main dietary source of LMOs, accompanied by higher bacterial LMOs intake and lower fungal intake. Therefore, based on these findings, further research is needed to determine whether the observed effects are driven primarily by the total dose of LMOs consumed, the predominance of bacteria over fungi or by the type of dietary source—particularly fermented products such as yogurt—and to clarify their potential protective role in relation to health biomarkers, including LDL and total cholesterol and the potential to counteract age-related declines in gut microbial functionality.

To summarize, this study demonstrates that the dietary intake of LMOs increases across adulthood and is predominantly derived from fermented dairy products. The LMOs consumption level was associated with age-dependent patterns in gut microbial composition, intestinal microbial metabolites, and host biochemical markers. Specifically, bacterial and fungal LMOs exhibited differential associations with key microbial taxa, SCFAs production, and biomarkers related to lipid metabolism and oxidative status. These findings support the hypothesis that dietary LMOs may contribute to the modulation of gut ecosystem functionality and host physiological processes throughout aging. Nevertheless, given the observational and cross-sectional design of our study, causal inferences cannot be established. Future longitudinal and controlled intervention studies integrating microbiome, metabolomic, and clinical assessments are warranted to disentangle the independent and synergistic effects of LMOs and other fermentation-derived bioactive constituents within complex food matrices and to identify the mechanisms of action involved. To this end, dietary assessment tools for a more refined characterization of LMOs intake, including microbial viability and product-specific compositional variability, are also needed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation12070308/s1, Table S1: General, anthropometric characteristics and biochemical parameters of the sample of study according to age groups; Table S2: Daily energy consumption and intake of the main food groups of the sample of study according to age groups.

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Abbreviations

The following abbreviations are used in this manuscript:

APB	Sum of acetic, propionic and butyric acids
BCFAs	Branched-chain fatty acids
BMI	Body mass index
CESNID	Centre for Higher Education in Nutrition and Dietetics
CFU	Colony forming units
CRP	C-reactive protein
FFQ	Food frequency questionnaire
HDL	High-density lipoprotein
IQR	Interquartile range
LDL	Low-density lipoprotein
LMOs	Live microorganisms
MDA	Malondialdehyde
MedHi	Food with medium or high content in live microorganisms
SCFAs	Short-chain fatty acids
SEEDO	Spanish Society for the Study of Obesity
qPCR	Quantitative polymerase chain reaction

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