medicina

MDPI

Article

The Effect of Synbiotic Supplementation on Uremic Toxins, Oxidative Stress, and Inflammation in Hemodialysis Patients—Results of an Uncontrolled Prospective Single-Arm Study

Teodor Kuskunov [1,2], Eduard Tilkiyan [3,4], Daniel Doykov [5,6], Krasimir Boyanov [7], Anelia Bivolarska [7] and Bozhidar Hristov [5,6,*]

1 Department of Propaedeutics of Internal Diseases, Medical Faculty, Medical University of Plovdiv, 4000 Plovdiv, Bulgaria; kuskunov@abv.bg
2 Hemodialysis Unit, University Hospital "Kaspela", 4000 Plovdiv, Bulgaria
3 Second Department of Internal Diseases, Section "Nephrology", Medical Faculty, Medical University of Plovdiv, 4000 Plovdiv, Bulgaria; eet64@yahoo.com
4 Nephrology Clinic, University Hospital "Kaspela", 4000 Plovdiv, Bulgaria
5 Second Department of Internal Diseases, Section "Gastroenterology", Medical Faculty, Medical University of Plovdiv, 4000 Plovdiv, Bulgaria; daniel_doykov@abv.bg
6 Gastroenterology Clinic, University Hospital "Kaspela", 4000 Plovdiv, Bulgaria
7 Department of Medical Biochemistry, Faculty of Pharmacy, Medical University of Plovdiv, 4000 Plovdiv, Bulgaria; krasimir.boyanov@mu-plovdiv.bg (K.B.); anelia.bivolarska@mu-plovdiv.bg (A.B.)
* Correspondence: hristov.bozhidar@abv.bg; Tel.: +359-88-4278187

Abstract: *Introduction*: Numerous studies to date have shown that the development of dysbiotic gut microbiota is a characteristic finding in chronic kidney disease (CKD). A number of uremic toxins progressively accumulate in the course of CKD, some of them generated by the intestinal microbiome, such as indoxyl sulfate (IS) and p-cresyl sulfate (p-CS). They are found to be involved in the pathogenesis of certain complications of uremic syndrome, including low-grade chronic inflammation and oxidative stress. The aim of the present study is to research the serum concentration of IS and p-CS in end stage renal disease (ESRD) patients undergoing conventional hemodialysis, as well as to study the possibilities of influencing some markers of inflammation and oxidative stress after taking a synbiotic. *Materials and Methods*: Thirty patients with end-stage renal disease (ESRD) undergoing hemodialysis treatment who were taking a synbiotic in the form of *Lactobacillus acidophilus* La-14 2 $\times$ 10^{11} (CFU)/g and prebiotic fructooligosaccharides were included in the study. Serum levels of total IS, total p-CS, Interleukin-6 (IL-6), and Malondialdehyde (MDA) were measured at baseline and after 8 weeks. *Results*. The baseline values of the four investigated indicators in the patients were significantly higher—p-CS (29.26 $\pm$ 58.32 pg/mL), IS (212.89 $\pm$ 208.59 ng/mL), IL-6 (13.84 $\pm$ 2.02 pg/mL), and MDA (1430.33 $\pm$ 583.42 pg/mL), compared to the results obtained after 8 weeks of intake, as we found a significant decrease in the parameters compared to the baseline—p-CS (6.40 $\pm$ 0.79 pg/mL, $p = 0.041$), IS (47.08 $\pm$ 3.24 ng/mL, $p < 0.001$), IL-6 (9.14 $\pm$ 1.67 pg/mL, $p < 0.001$), and MDA (1003.47 $\pm$ 518.37 pg/mL, $p < 0.001$). *Conclusions*: The current study found that the restoration of the intestinal microbiota in patients with CKD significantly decreases the level of certain uremic toxins. It is likely that this favorably affects certain aspects of CKD, such as persistent low-grade inflammation and oxidative stress.

Keywords: chronic kidney disease; hemodialysis; indoxyl sulfate; p-cresyl sulfate; interleukin-6; malondialdehyde; synbiotic

check for updates

Citation: Kuskunov, T.; Tilkiyan, E.; Doykov, D.; Boyanov, K.; Bivolarska, A.; Hristov, B. The Effect of Synbiotic Supplementation on Uremic Toxins, Oxidative Stress, and Inflammation in Hemodialysis Patients—Results of an Uncontrolled Prospective Single-Arm Study. *Medicina* **2023**, *59*, 1383. https://doi.org/10.3390/medicina59081383

Academic Editor: Michal Nowicki

Received: 11 June 2023
Revised: 24 July 2023
Accepted: 24 July 2023
Published: 28 July 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The human gut microbiota consists of over 100 trillion microbial cells, and its species diversity is unique to each individual and includes 500 to 1000 bacterial species [1,2].

Different parts of the gastrointestinal tract are colonized in varying quantitative proportions, with the predominant bacterial strains being *Bacteroidetes*, *Actinobacteria*, and *Frimicutes* [3,4]. In their study, The Human Microbiome Project, Gevers et al. [5] reveal the vital importance and impact of the gut microbiome on human health and disease development.

The progression of chronic kidney disease (CKD) to end-stage renal disease (ESRD) is associated with both a qualitative and a quantitative change in the composition of the intestinal microbiota, which leads to the development of a dysbiotic intestinal condition in these patients [6–9]. In 1996, Simenhoff et al. [6] reported that patients with uremia had a significantly higher number of aerobic and anaerobic micro-organisms inhabiting the duodenum and small intestine compared to healthy individuals. Hida et al. [7] demonstrated an increased number of aerobic bacteria from *the Enterobacteria* and *Enterococci families* combined with a decreased count of *Bifidobacterium species* in hemodialysis patients, as well as much higher *Clostridium perfringens* colonization in the same patients. Later, Vaziri et al. [8] reported a significantly higher abundance of *Brachybacterium*, *Catenibacterium*, *Halomonadaceae*, *Enterobacteriaceae*, *Moraxellaceae*, *Polyangiaceae*, *Thiothrix*, *Nesterenkonia*, and *Pseudomonadaceae* families in ESRD patients compared to healthy controls. A distinctive feature of some of these micro-organisms is a pronounced urease and uricase activity, as well as increased synthesis of indoles and phenols [9]. This, on its behalf, leads to an increased production of toxic substances and their subsequent accumulation in the blood circulation, due to reduced renal clearance in these patients [10,11]. Additionally, Wong et al. [9] indicated that the intestinal dysbiosis in CKD causes an impaired synthesis of useful metabolites such as short-chain fatty acids (SCFA), which are the main source of nutrition for intestinal bacterial proliferation.

To date, more than 150 molecules regarded as toxic in the context of uremia have been discovered [12]. Some of them are classified as protein-bound uremic toxins, including indoxyl sulfate and p-cresyl sulfate [13]. The aforementioned are products of the intestinal microbial metabolism of the aromatic amino acids—tyrosine, phenylalanine, and tryptophan—and accumulate progressively in the course of CKD [14–16]. Tryptophan is metabolized mainly into indole, while tyrosine and phenylalanine are metabolized into para-cresol, both of which are eventually absorbed and undergo partial detoxification in the liver mainly by sulfation to indoxyl sulfate (IS) and para-cresyl sulfate (p-CS), respectively [17].

In preserved renal function, the predominant mechanism of excretion of p-CS and IS in the urine is through tubular secretion [18,19]. In patients undergoing conventional hemodialysis treatment, however, their elimination is largely limited due to their high affinity to plasma proteins [20]. High serum concentrations of IS and p-CS in CKD have a variety of detrimental effects on the normal function of multiple organs and systems. Elevated serum IS levels are associated with numerous negative effects on the cardiovascular system including increased oxidative stress on the vascular endothelium, decreased vascular elasticity and vascular smooth muscle proliferation, aortic calcification, and increased cardiovascular-associated and total mortality in CKD patients [21–23]. Lin et al. [24] reported that IS and p-CS increase the risk of peripheral vascular disease and vascular access route thrombosis in hemodialysis patients. The role of IS in the pathophysiology of bone disorders in CKD is associated with the suppression of osteoclast activity and development of adynamic bone disease [25,26]. Nii-Kono et al. [27] hypothesize that IS stimulates oxidative stress in osteoblasts and promote the development of resistance to parathormone, leading to a reduced rate of bone formation. In another study of theirs, Lin et al. [28] establish a positive association between high serum levels of IS and those of fibroblast growth factor 23 (FGF-23). IS has been found to have a marked profibrotic effect on the myocardium, to stimulate myocardiocyte hypertrophy and to increase the risk of atrial fibrillation [29–31]. A correlation has been found between IS levels and the development of anemic syndrome in patients with CKD, as it inhibits erythropoiesis, suppresses the activity of erythropoietin, and potentiates eryptosis—the programmed cell death of erythrocytes [32–34]. Similar to IS, elevated serum levels of p-CS increase the risk

of cardiovascular and total mortality in patients with CKD [35,36]. P-CS also stimulates profibrotic and inflammatory responses as well as oxidative stress [37–39].

2. Materials and Methods

2.1. Study Settings and Population

Thirty patients undergoing chronic hemodialysis treatment in the Hemodialysis Unit of University Hospital Kaspela, Plovdiv, Bulgaria were included in the study. The laboratory analysis of the samples was carried out in the Department of Medical Biochemistry of the Medical University of Plovdiv. The study was approved by the scientific ethics committee of Medical University of Plovdiv (protocol No. 1/19.01.2023) and all subjects gave written informed consent. Design: Prospective, quasi-experimental single-center study.

We tested the serum levels of indoxyl sulfate, p-cresyl sulfate, malondialdehyde, and interleukin-6 in 30 patients (women $n = 13$, men $n = 17$) undergoing conventional hemodialysis treatment at baseline and after taking a synbiotic for 8 weeks. Inclusion criteria were as follows: age over 18 years, chronic hemodialysis treatment, and ability to obtain adequate informed consent. Failure to obtain informed consent, having an active inflammatory disease, as well as intake of medications affecting the intestinal microbiome were regarded as exclusion criteria. Patients who had undergone antibiotic or chemotherapy in the previous three months, or were treated with biological agents during the same period, as well as taking phosphate binders, statins, and proton pump inhibitors were excluded from study.

Hemodialysis procedures were performed in line with the standard protocol using polyethersulfone high-flux dialyzer for each dialysis session. The applied synbiotic consists of 75 mg *Lactobacillus acidophilus* La-14 2×10^{11} CFU/g and 65 mg prebiotic fructooligosaccharides, taken once a day, 1 h after a meal. Patients were instructed not to alter their dietary habits, physical activities, or medication regimens. At the beginning of the study and again 8 weeks later, 5 mL of blood was drawn from each patient after an 8 h fasting. Blood samples were allowed to clot at room temperature and then centrifuged at $1000\times g$ for 20 min. The serum was separated into Eppendorf microtubes and frozen at -80 °C for subsequent use.

2.2. Laboratory Analysis

Determination of serum concentrations of IS, para-CS, IL-6, and MDA was performed by enzyme-linked immunosorbent assay (ELISA). The assay was performed using commercial kits according to the manufacturer's protocol (ELK Biotechnology, PRC; Denver, CO, USA).

2.3. Statistical Analysis

Statistical analyzes were performed using Statistical Package for Social Sciences (SPSS) Ver. 23 (SPSS, Inc., Chicago, IL, USA). All quantitative parameters were analyzed using Kolmogorov–Smirnov test. Unpaired and paired *t*-test were used to compare parameters between and within groups, respectively. Results were expressed as mean SD and differences were considered significant at $p < 0.05$.

3. Results

A total of 30 patients, 17 males and 13 females, were included in the study. No statistically significant difference was found based on sex ($p = 0.638$). The mean age of the subjects was 62.6 ± 14.3 years, again without any significant difference between men (60.4 ± 12.3 years) and women (63.1 ± 15.5 years) ($p = 0.743$).

In the Table 1 is illustrated the distribution of the studied population based on the frequency the dialysis procedures per week. The majority of patients on renal replacement therapy performed dialysis treatment three times a week with a duration of 4 h ($n = 21$). Due to preserved residual renal function, five of the patients are dialyzed twice a week for 4 h. The remaining four of patients were on a hemodialysis regimen three times a week with a duration of 3 h and 30 min.

Table 1. Distribution of patients undergoing hemodialysis treatment according to the frequency of therapy.

Weekly Frequency and Duration of the Hemodialysis Procedure	Number of Patients
3 × 4 h	21
3 × 3 h and 30 min	4
2 × 4 h	5

Regarding the reasons for developing ESRD, the number of patients with chronic glomerulonephritis is the largest. Next are the patients with diabetic nephropathy and those with hypertensive nephroangiosclerosis (Table 2). As comorbidities, apart from secondary anemia and hyperparathyreoidism, more than half of the patients (n = 17) have only arterial hypertension. One third of them (n = 10) have diabetes and hypertension and three of the patients have hypertension and coronary heart disease as concomitant diseases. Nine patients had history of a cerebrovascular or cardiovascular event. The largest part of the patients (n = 14) have been undergoing hemodialisys treatment for more than 3 years, a little over 1/3 (n = 11) have been included in a dialysis program within the last year for at least 3 months, and the rest of the patients (n = 5) received renal replacement therapy for 1 to 3 years.

Table 2. Distribution of patients undergoing hemodialysis treatment according to etiology of ESRD.

Kidney Disease	Number of Patients
Chronic glomerulonephritis	13
Diabetic nephropathy	6
Hypertensive nephroangiosclerosis	6
Autosomal dominant polycystic disease	3
Other	2

In the Tables 3 and 4 are presented descriptive statistics of the studied indicators, before and after taking a synbiotic, in patients undergoing hemodialysis treatment. The statistical significance of the obtained results is also reflected. The serum levels of the two uremic toxins—IS and p-CS (Table 3 and Figures 1 and 2)—decreased significantly after 8 weeks of synbiotic intake ($p < 0.001$ and $p = 0.041$). Similar results were established in the serum concentrations of MDA and IL-6 (Table 4 and Figures 3 and 4) at the end of week 8 compared to the baseline levels ($p < 0.001$ and $p < 0.001$).

Table 3. IS and p-CS levels at baseline and at week 8.

	Indoxyl Sulfate ng/mL (Baseline)	Indoxyl Sulfate ng/mL (Week 8)	p-Cresyl Sulfate pg/mL (Baseline)	p-Cresyl Sulfate pg/mL (Week 8)
mean ± SD	212.89 ± 208.59	47.08 ± 3.24	29.26 ± 58.32	6.40 ± 0.79
95% confidence intervals	135.00–290.78	45.87–48.30	7.48–51.04	6.10–6.70
min.	42.48	40.94	6.17	6.17
max.	1012.28	54.14	265.00	8.43
SEM	38.08	0.59	10.64	0.14
p-value	$p < 0.001$ (paired-samples t-test)		$p = 0.041$ (paired-samples t-test)	

SEM—standard error of mean.

Table 4. IL-6 and Malondialdehyde (MDA) levels at baseline and at week 8.

	IL-6 pg/mL Before Taking a Synbiotic	IL-6 pg/mL after Intake	MDA pg/mL before Intake	MDA pg/mL after Intake
mean ± SD	13.84 ± 2.02	9.14 ± 1.67	1430.33 ± 583.42	1003.47 ± 518.37
95% confidence intervals	13.08–14.59	8.52–9.77	1212.48–1648.19	809.91–1197.04
min.	9.32	6.19	7.07	114.58
max.	22.46	11.72	2227.85	2046.74
SEM	0.37	0.30	106.51	94.64
p-value	$p < 0.001$ (paired-samples *t*-test)		$p < 0.001$ (paired-samples *t*-test)	

MDA—malondialedhyde, IL-6—interleukin-6.

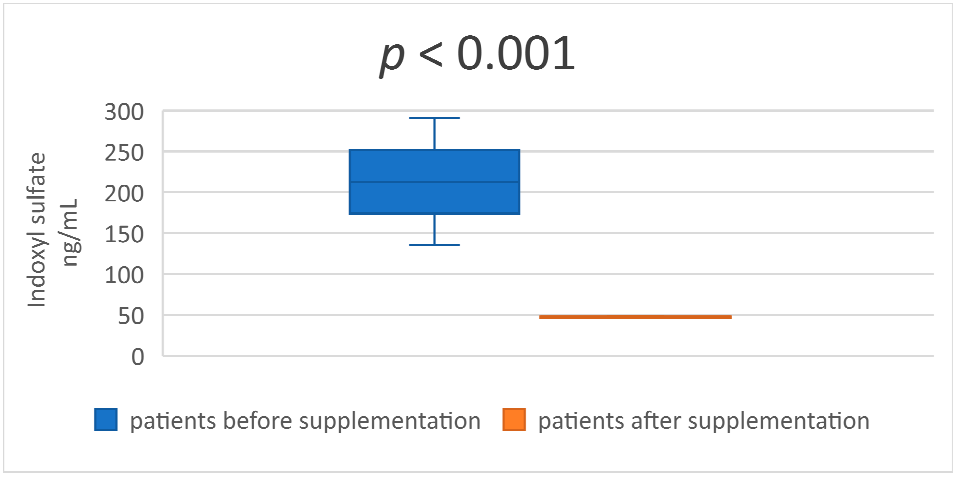


Figure 1. Mean value and confidence interval (CI) of serum indoxyl sulfate before and after supplementation.

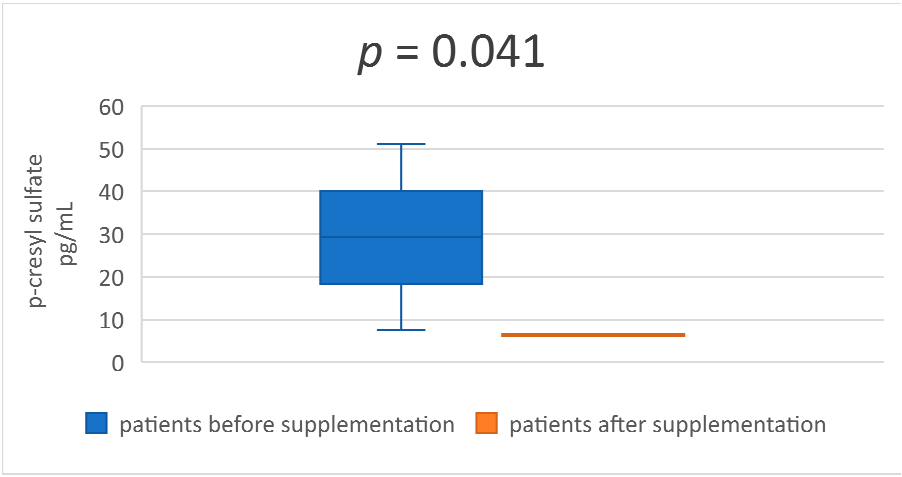


Figure 2. Mean value and CI of serum p-sresyl sulfate before and after supplementation.

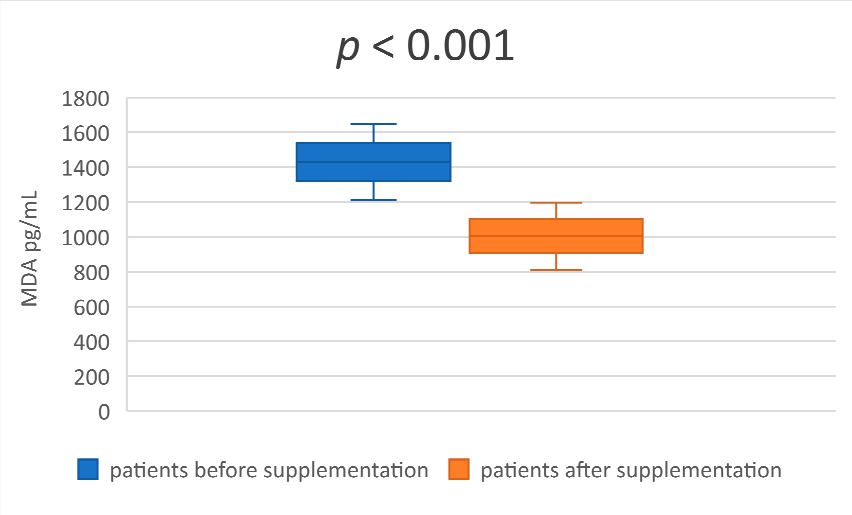


Figure 3. Mean value and CI of serum MDA before and after supplementation.

Table 5 represent the correlation between the evaluated uremic toxins prior and post-treatment and the frequency and duration of dialysis procedure.

Table 5. Values of IS, p-CS, IL-6, and MDA in the three subgroups of hemodialysis patients according to the frequency and length of the dialysis treatment.

Weekly Frequency and Duration of HD Procedure	Parameters Before and After Synbiotic Intake	*n*	Mean ± SD	*p*-Value *
3 × 4 h	IS ng/mL before IS ng/mL after	21	159.88 ± 150.925 47.56 ± 3.38	0.003
	p-CS pg/ml before p-CS pg/mL after	21	14.3 ± 7.74 6.25 ± 0.66	0.001
	IL-6 pg/mL before IL-6 pg/mL after	21	13.65 ± 1.26 9.16 ± 1.78	0.001
	MDA pg/mL before MDA pg/mL after	21	1307.09 ± 617.18 422.37 ± 592.07	0.001
3 × 3 h and 30 min	IS ng/mL before IS ng/mL after	4	407.19 ± 406.44 47.40 ± 2.41	0.176
	p-CS pg/ml before p-CS pg/mL after	4	16.64 ±7.14 6.28 ± 0.46	0.058
	IL-6 pg/mL before IL-6 pg/mL after	4	15.05 ± 5.09 8.82 ± 1.51	0.109
	MDA pg/mL before MDA pg/mL after	4	1657.88 ± 529.77 163.42 ± 326.85	0.012
2 × 4 h	IS ng/mL before IS ng/mL after	5	280.10 ± 139.68 44.84 ± 2.72	0.019
	p-CS pg/ml before p-CS pg/mL after	5	102.23 ± 127.81 7.14 ± 1.19	0.173
	IL-6 pg/mL before IL-6 pg/mL after	5	13.66 ± 0.80 9.32 ± 1.57	0.002
	MDA pg/mL before MDA pg/mL after	5	1765.90 ± 283.70 1047.47 ± 976.30	0.191

*—paired-samples *t*-test.

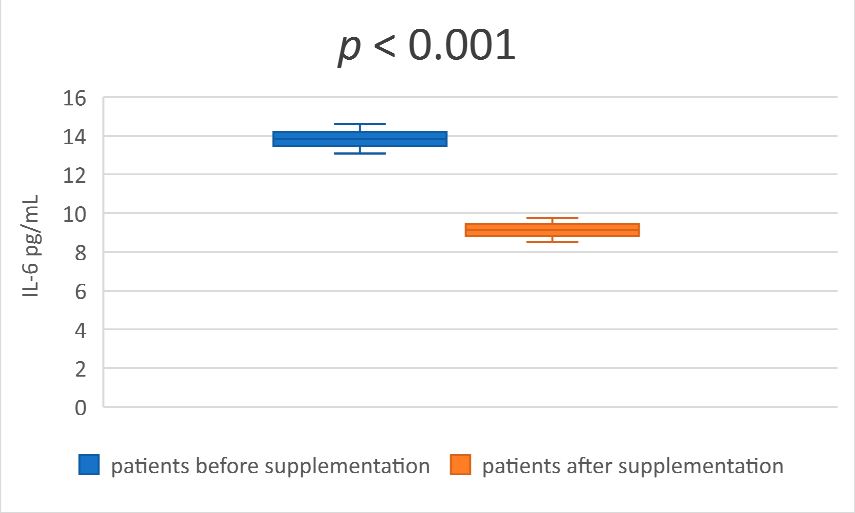


Figure 4. Mean value and CI of serum IL-6 before and after supplementation.

Table 6 shows the values of certain blood parameters that we examine routinely in these patients. After two months of synbiotic supplementation, we found a significant difference (but within the normal range) in serum levels of total protein, potassium, and leukocyte count.

Table 6. Serum levels of some blood parameters before and after synbiotic intake in hemodialysis patients.

Blood Parameters	Mean	*n*	Std. Deviation	Std. Error Mean	*p*-Value *
HGB g/L before intake HGB g/L after intake	102.56 100.30	30	15.35 17.46	2.80 3.18	0.293
RBC 10^{12}/L before intake RBC 10^{12}/L after intake	3.45 3.33	30	0.59 0.69	0.10 0.10	0.142
WBC 10^{9}/L before intake WBC 10^{9}/L after intake	8.54 7.62	30	3.17 2.07	0.57 0.37	0.05
PLT 10^{9}/L before intake PLT 10^{9}/L after intake	218.9 214.9	30	120.17 103.2	21.9 18.84	0.711
Urea mmol/L before intake Urea mmol/L after intake	22.97 21.5	30	5.55 3.51	1.01 0.64	0.068
Creatinine µmol/L before intake Creatinine µmol/L after intake	743.5 720.6	30	224.11 214.45	40.91 39.15	0.136
T.protein g/L before intake T.protein g/L after intake	68.33 66.46	30	6.77 5.8	1.23 1.06	0.025
K mmol/L before intake K mmol/L after intake	5.45 5.2	30	0.85 0.88	0.15 0.16	0.031
T.Ca mmol/L before intake T.Ca mmol/L after intake	2.31 2.29	30	0.22 0.23	0.04 0.04	0.239
P mmol/L before intake P mmol/L after intake	1.86 1.96	30	0.54 0.53	0.09 0.09	0.339
Fe µmol/L before intake Fe µmol/L after intake	8.54 9.12	30	2.91 2.8	0.53 0.51	0.294

*—paired-samples *t*-test.

No adverse events were reported. All enrolled patients completed the study and were subjected to analysis.

4. Discussion

The current paper outlines a strong positive correlation between the intake of pro-, pre-, and synbiotics, and the metabolism of cresols and indoles. Such results are largely in

line with the existing evidence on the matter [40,41]. In their systematic review, Nguyen TTU et al. [41] summarize a number of studies that demonstrate the positive impact of the gut microbiota modulators on the serum levels of p-cresyl sulfate in hemodialysis patients. They noted the insufficient number of and the need for further studies related to tracking serum levels of indoxyl sulfate in hemodialysis patients after probiotic or synbiotic supplementation. A significant association between dysbiotic gut microbiota and low-grade systemic inflammation in end-stage CKD patients has been demonstrated [42]. The pro-inflammatory biomarker IL-6, which was significantly decreased after taking a synbiotic in our study, appears to be the best predictor of cardiovascular risk and all-cause mortality in CKD [43]. Rossi et al. [44] found a correlation between increased values of IS and p-CS and increased pro-inflammatory biomarkers such as interleukin-6 and glutathione peroxidase in patients with CKD.

The patients on hemodialysis are subjected to enhanced oxidative stress as a result of elevated pro-oxidant activity [45] and inefficient antioxidant systems [46] related to the end stage of the renal disease as well as with the techniques of the hemodialysis treatment [47,48]. It is considered that the malondialdehyde generated from lipid peroxidation is a biomarker for increased oxidative stress in CKD [49], and its serum concentration in CKD patients is higher than that of healthy individuals [50,51]. Several studies have reported a potential antioxidant effect and decrease in oxidative stress biomarkers following therapeutic modulation of the gut microbiota. The systematic review by Nguyen TTU et al. [41] is also the first summary analysis of all studies related to monitoring MDA values in hemodialysis patients to date. In six of them, the beneficial effect of supplementation with probiotic or synbiotic on the serum levels of this oxidative stress marker was confirmed. The same effect was observed on serum concentrations of some of the inflammatory markers, including IL-6. The presented results are largely similar to the ones obtained in our study and support the conclusion derived by our research that the modulation of intestinal microbiota might have a positive effect on the levels of inflammatory biomarkers in patients with ESRD.

Despite the relatively small number of patients in our study, we analyzed the results obtained in the three subgroups depending on the frequency and the length of hemodialysis procedures (Table 5). It is noteworthy that the baseline values of protein-bound uremic toxins were lower in patients undergoing hemodialysis treatment three times a week for 4 h compared to the other two subgroups. In addition, achieving a significant reduction in IS and p-CS after synbiotic supplementation in patients with a lower weekly dialysis dose was more difficult than in patients undergoing hemodialysis treatment with a duration of 12 h per week. A similar finding was also observed when evaluating the serum levels of IL-6 and MDA. These results suggest that, although low, the clearance of protein-bound uremic toxins across the dialysis membrane is still of essence and the higher weekly dialysis dose leads to lower levels of their serum concentrations.

We also evaluated the values of IS and p-CS depending on the history of cerebrovascular or cardiovascular events in the study population. In patients with stroke or heart attack (n = 9), the baseline values of IS (248.87 $\pm$ 316.97 ng/mL) were higher compared to patients without such diseases (n = 21, 97.47 $\pm$ 148.55 ng/mL). We did not find the same correlation regarding baseline serum p-CS in patients with heart attack or stroke (13.03 $\pm$ 4.40 pg/mL) versus those without a cerebrovascular or cardiovascular event (36.22 $\pm$ 68.96 pg/mL).

In addition to the above-mentioned results related to our study, the tendency towards a decrease in serum creatinine levels (743.5 $\pm$ 224.11; 720,6 $\pm$ 214.45 µmol/L; p = 0.36) and urea (22.97 $\pm$ 5.55; 21.5 $\pm$ 3.51 mmol/L; p = 0.068) after synbiotic supplementation should be noted, although they did not reach significant values (Table 6).

It should be noted, though, that not all authors confirm the positive effect of the therapeutic modulation of the intestinal microbiota on the serum concentrations of biomarkers of inflammation and oxidative stress. For example, Hatakka et al. [52] did not find a significant decrease in CRP after taking a probiotic in patients with rheumatoid arthritis, and Lamprecht et al. [53] also did not find any effect of probiotic administration on serum

MDA and IL-6 levels in trained men. The presence of such results is probably related to differences in the probiotic strains used, the applied probiotic dose, and the specificity of the dosage form taken.

The main disadvantages of the current study are the small population size and the lack of a control group to outline the causal relation between the medication intake and the decrease of toxin levels. The supplementation time probably also could be regarded as suboptimal. The parameters were measured only twice—at the beginning of the study and after 8 weeks. On the positive side we should outline the prospective nature of the research and the fact that there were no patients lost for follow-up, which undeniably increases the credibility of the results.

5. Conclusions

Synbiotic supplementation in patients with CKD undergoing conventional hemodialysis treatment leads to a decrease in the plasma levels of IS and p-CS. This has a beneficial effect on some of the complications of CKD, such as low-grade chronic inflammation and oxidative stress with a decrease in the serum concentrations of IL-6 and MDA. A high weekly dialysis dose of 12 h maintains lower serum levels of gut-delivered protein-bound toxins in hemodialysis patients despite their low clearance across the dialysis membrane. Additional multicenter studies are needed to specify the type, composition, and dose of the supplement used in patients with CKD. Although there is still no consensus regarding the duration and regimen of their intake, synbiotic supplementation can be used as an additional option in the treatment of hemodialysis patients.

Author Contributions: Conceptualization, E.T. and T.K.; methodology, A.B. and K.B.; formal analysis, A.B. and K.B.; investigation, T.K.; resources, D.D. and B.H.; data curation, E.T.; writing—original draft preparation, T.K.; writing—review and editing, B.H.; visualization, B.H.; supervision, E.T. and D.D.; project administration, T.K.; funding acquisition, D.D. All authors have read and agreed to the published version of the manuscript.

Funding: The study is related to DPDP project 05/2022 on the topic "Serum level of indoxyl sulfate and para-cresol in patients with CKD IV-V stage as biochemical markers for intestinal dysbiosis in uremia—clinical significance and possibilities for therapeutic control", financed by the program "Doctoral and postdoctoral projects 2022" from Medical University of Plovdiv.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Medical University of Plovdiv (protocol No. 1/19.01.2023) for studies involving humans.

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

Data Availability Statement: The data presented in this study are available upon request from the corresponding author. The data are not publicly available due to national legal restrictions.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

CKD	chronic kidney disease
IS	indoxyl sulfate
p-CS	p-cresyl sulfate
ESRD	end-stage renal disease
CFU	colony forming units
ELISA	enzyme-linked immunosorbent assay
SEM	standard error of mean
IL	interleukin 6
MDA	malondialdehyde
SCFA	short-chain fatty acids
FGF-23	fibroblast growth factor 23
TNF-α	tumor necrosis factor–alpha
CRP–C	reactive protein

References

1. Ramezani, A.; Raj, D.S. The Gut Microbiome, Kidney Disease, and Targeted Interventions. *J. Am. Soc. Nephrol.* **2014**, *25*, 657–670. [CrossRef]
2. Xu, J.; Gordon, J.I. Honor thy symbionts. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 10452–10459. [CrossRef]
3. Eckburg, P.B.; Bik, E.M.; Bernstein, C.N.; Purdom, E.; Dethlefsen, L.; Sargent, M.; Gill, S.R.; Nelson, K.E.; Relman, D.A. Diversity of the human intestinal microbial flora. *Science* **2005**, *308*, 1635–1638. [CrossRef]
4. Tremaroli, V.; Bäckhed, F. Functional interactions between the gut microbiota and host metabolism. *Nature* **2012**, *489*, 242–249. [CrossRef]
5. Gevers, D.; Knight, R.; Petrosino, J.F.; Huang, K.; McGuire, A.L.; Birren, B.W.; Nelson, K.E.; White, O.; Methé, B.A.; Huttenhower, C. The Human Microbiome Project: A community resource for the healthy human microbiome. *PLoS Biol.* **2012**, *10*, e1001377. [CrossRef]
6. Simenhoff, M.L.; Dunn, S.R.; Zollner, G.P.; Fitzpatrick, M.E.; Emery, S.M.; Sandine, W.E.; Ayres, J.W. Biomodulation of the toxic and nutritional effects of small bowel bacterial overgrowth in end-stage kidney disease using freeze-dried Lactobacillus acidophilus. *Min. Electrolyte Metab.* **1996**, *22*, 92–96.
7. Hida, M.; Aiba, Y.; Sawamura, S.; Suzuki, N.; Satoh, T.; Koga, Y. Inhibition of the accumulation of uremic toxins in the blood and their precursors in the feces after oral administration of Lebenin, a lactic acid bacteria preparation, to uremic patients undergoing hemodialysis. *Nephron* **1996**, *74*, 349–355. [CrossRef]
8. Vaziri, N.D.; Wong, J.; Pahl, M.; Piceno, Y.M.; Yuan, J.; DeSantis, T.Z.; Ni, Z.; Nguyen, T.-H.; Andersen, G.L. Chronic kidney disease alters intestinal microbial flora. *Kidney Int.* **2013**, *83*, 308–315. [CrossRef] [PubMed]
9. Wong, J.; Piceno, Y.M.; DeSantis, T.Z.; Pahl, M.; Andersen, G.L.; Vaziri, N.D. Expansion of urease- and uricase-containing, indole and p-cresol-forming and contraction of short-chain fatty acid-producing intestinal microbiota in ESRD. *Am. J. Nephrol.* **2014**, *39*, 230–237. [CrossRef]
10. Ramezani, A.; Massy, Z.A.; Meijers, B.; Evenepoel, P.; Vanholder, R.; Raj, D.S. Role of the Gut Microbiome in Uremia: A Potential Therapeutic Target. *Am. J. Kidney Dis.* **2016**, *67*, 483–498. [CrossRef]
11. Meyer, T.W.; Hostetter, T.H. Uremia. *N. Engl. J. Med.* **2007**, *357*, 1316–1325. [CrossRef] [PubMed]
12. Duranton, F.; Cohen, G.; De Smet, R.; Rodriguez, M.; Jankowski, J.; Vanholder, R.; Argiles, A.; European Uremic Toxin Work Group. Normal and pathologic concentrations of uremic toxins. *J. Am. Soc. Nephrol.* **2012**, *23*, 1258–1270. [CrossRef] [PubMed]
13. Chen, Y.Y.; Chen, D.Q.; Chen, L.; Liu, J.R.; Vaziri, N.D.; Guo, Y.; Zhao, Y.Y. Microbiome–metabolome reveals the contribution of gut–kidney axis on kidney disease. *J. Transl. Med.* **2019**, *17*, 5. [CrossRef]
14. Vanholder, R.; Glorieux, G.; De Smet, R.; Lameire, N. New insights in uremic toxins. *Kidney Int.* **2003**, *63*, S6–S10. [CrossRef]
15. Gryp, T.; Vanholder, R.; Vaneechoutte, M.; Glorieux, G. p-Cresyl Sulfate. *Toxins* **2017**, *9*, 52. [CrossRef]
16. Mair, R.D.; Sirich, T.L.; Plummer, N.S.; Meyer, T.W. Characteristics of Colon-Derived Uremic Solutes. Clin. *J. Am. Soc. Nephrol.* **2018**, *13*, 1398–1404. [CrossRef]
17. Vanholder, R.; De Smet, R.; Glorieux, G.; Argilés, A.; Baurmeister, U.; Brunet, P.; Clark, W.; Cohen, G.; De Deyn, P.P.; Deppisch, R.; et al. Review on uremic toxins: Classification, concentration, and interindividual variability. *Kidney Int.* **2003**, *63*, 1934–1943. [CrossRef]
18. Suchy-Dicey, A.M.; Laha, T.; Hoofnagle, A.; Newitt, R.; Sirich, T.L.; Meyer, T.W.; Thummel, K.E.; Yanez, N.D.; Himmelfarb, J.; Weiss, N.S.; et al. Tubular Secretion in CKD. *J. Am. Soc. Nephrol.* **2016**, *27*, 2148–2155. [CrossRef]
19. Masereeuw, R.; Mutsaers, H.A.; Toyohara, T.; Abe, T.; Jhawar, S.; Sweet, D.H.; Lowenstein, J. The kidney and uremic toxin removal: Glomerulus or tubule? *Semin. Nephrol.* **2014**, *34*, 191–208. [CrossRef]
20. Palmer, S.C.; Rabindranath, K.S.; Craig, J.C.; Roderick, P.J.; Locatelli, F.; Strippoli, G.F. High-flux versus low-flux membranes for end-stage kidney disease. *Cochrane Database Syst. Rev.* **2012**, *2012*, CD005016. [CrossRef]
21. Barreto, F.C.; Barreto, D.V.; Liabeuf, S.; Meert, N.; Glorieux, G.; Temmar, M.; Choukroun, G.; Vanholder, R.; Massy, Z.A.; European Uremic Toxin Work Group (EUTox). Serum indoxyl sulfate is associated with vascular disease and mortality in chronic kidney disease patients. *Clin. J. Am. Soc. Nephrol.* **2009**, *4*, 1551–1558. [CrossRef] [PubMed]
22. Yamamoto, H.; Tsuruoka, S.; Ioka, T.; Ando, H.; Ito, C.; Akimoto, T.; Fujimura, A.; Asano, Y.; Kusano, E. Indoxyl sulfate stimulates proliferation of rat vascular smooth muscle cells. *Kidney Int.* **2006**, *69*, 1780–1785. [CrossRef] [PubMed]
23. Dou, L.; Jourde-Chiche, N.; Faure, V.; Cerini, C.; Berland, Y.; Dignat-George, F.; Brunet, P. The uremic solute indoxyl sulfate induces oxidative stress in endothelial cells. *J. Thromb. Haemost.* **2007**, *5*, 1302–1308. [CrossRef]
24. Lin, C.-J.; Pan, C.-F.; Liu, H.-L.; Chuang, C.-K.; Jayakumar, T.; Wang, T.-J.; Chen, H.-H.; Wu, C.-J. The role of protein-bound uremic toxins on peripheral artery disease and vascular access failure in patients on hemodialysis. *Atherosclerosis* **2012**, *225*, 173–179. [CrossRef] [PubMed]
25. Mozar, A.; Louvet, L.; Godin, C.; Mentaverri, R.; Brazier, M.; Kamel, S.; Massy, Z.A. Indoxyl sulphate inhibits osteoclast differentiation and function. *Nephrol. Dial. Transplant.* **2012**, *27*, 2176–2181. [CrossRef]
26. Barreto, F.C.; Barreto, D.V.; Canziani, M.E.F.; Tomiyama, C.; Higa, A.; Mozar, A.; Glorieux, G.; Vanholder, R.; Massy, Z.A.; De Carvalho, A.B. Association between indoxyl sulfate and bone histomorphometry in pre-dialysis chronic kidney disease patients. *Braz. J. Nephrol.* **2014**, *36*, 289–296. [CrossRef]

27. Nii-Kono, T.; Iwasaki, Y.; Uchida, M.; Fujieda, A.; Hosokawa, A.; Motojima, M.; Yamato, H.; Kurokawa, K.; Fukagawa, M. Indoxyl sulfate induces skeletal resistance to parathyroid hormone in cultured osteoblastic cells. *Kidney Int.* **2007**, *71*, 738–743. [CrossRef]
28. Lin, C.-J.; Pan, C.-F.; Chuang, C.-K.; Liu, H.-L.; Sun, F.-J.; Wang, T.-J.; Chen, H.-H.; Wu, C.-J. Association of Indoxyl Sulfate With Fibroblast Growth Factor 23 in Patients With Advanced Chronic Kidney Disease. *Am. J. Med. Sci.* **2014**, *347*, 370–376. [CrossRef]
29. Guldris, S.C.; Parra, E.G.; Amenós, A.C. Gut microbiota in chronic kidney disease. *Nefrología* **2017**, *37*, 9–19. [CrossRef]
30. Lekawanvijit, S.; Adrahtas, A.; Kelly, D.J.; Kompa, A.R.; Wang, B.H.; Krum, H. Does indoxyl sulfate, a uraemic toxin, have direct effects on cardiac fibroblasts and myocytes? *Eur. Heart J.* **2010**, *31*, 1771–1779. [CrossRef]
31. Aoki, K.; Teshima, Y.; Kondo, H.; Saito, S.; Fukui, A.; Fukunaga, N.; Nawata, T.; Shimada, T.; Takahashi, N.; Shibata, H. Role of Indoxyl Sulfate as a Predisposing Factor for Atrial Fibrillation in Renal Dysfunction. *J. Am. Heart Assoc.* **2015**, *4*, e002023. [CrossRef]
32. Chiang, C.-K.; Tanaka, T.; Inagi, R.; Fujita, T.; Nangaku, M. Indoxyl sulfate, a representative uremic toxin, suppresses erythropoietin production in a HIF-dependent manner. *Lab. Investig.* **2011**, *91*, 1564–1571. [CrossRef] [PubMed]
33. Nangaku, M.; Mimura, I.; Yamaguchi, J.; Higashijima, Y.; Wada, T.; Tanaka, T. Role of Uremic Toxins in Erythropoiesis-Stimulating Agent Resistance in Chronic Kidney Disease and Dialysis Patients. *J. Ren. Nutr.* **2015**, *25*, 160–163. [CrossRef] [PubMed]
34. Ahmed, M.S.E.; Abed, M.; Voelkl, J.; Lang, F. Triggering of suicidal erythrocyte death by uremic toxin indoxyl sulfate. *BMC Nephrol.* **2013**, *14*, 244. [CrossRef] [PubMed]
35. Bammens, B.; Evenepoel, P.; Keuleers, H.; Verbeke, K.; Vanrenterghem, Y. Free serum concentrations of the protein-bound retention solute p-cresol predict mortality in hemodialysis patients. *Kidney Int.* **2006**, *69*, 1081–1087. [CrossRef]
36. Meijers, B.K.; Claes, K.; Bammens, B.; de Loor, H.; Viaene, L.; Verbeke, K.; Kuypers, D.R.; Vanrenterghem, Y.; Evenepoel, P. p-Cresol and cardiovascular risk in mild-to-moderate kidney disease. Clin. *J. Am. Soc. Nephrol.* **2010**, *5*, 1182–1189. [CrossRef] [PubMed]
37. Sato, E.; Mori, T.; Mishima, E.; Suzuki, A.; Sugawara, S.; Kurasawa, N.; Saigusa, D.; Miura, D.; Morikawa-Ichinose, T.; Saito, R.; et al. Metabolic alterations by indoxyl sulfate in skeletal muscle induce uremic sarcopenia in chronic kidney disease. *Sci. Rep.* **2016**, *6*, 36618. [CrossRef]
38. Vanholder, R.; Schepers, E.; Pletinck, A.; Nagler, E.V.; Glorieux, G. The uremic toxicity of indoxyl sulfate and p-cresyl sulfate: A systematic review. *J. Am. Soc. Nephrol.* **2014**, *25*, 1897–1907. [CrossRef]
39. Mishima, E.; Fukuda, S.; Kanemitsu, Y.; Saigusa, D.; Mukawa, C.; Asaji, K.; Matsumoto, Y.; Tsukamoto, H.; Tachikawa, T.; Tsukimi, T.; et al. Canagliflozin reduces plasma uremic toxins and alters the intestinal microbiota composition in a chronic kidney disease mouse model. *Am. J. Physiol. Physiol.* **2018**, *315*, F824–F833. [CrossRef]
40. March, D.S.; Jones, A.; Bishop, N.C.; Burton, J. The Efficacy of Prebiotic, Probiotic, and Synbiotic Supplementation in Modulating Gut-Derived Circulatory Particles Associated With Cardiovascular Disease in Individuals Receiving Dialysis: A Systematic Review and Meta-analysis of Randomized Controlled Trials. *J. Ren. Nutr.* **2020**, *30*, 347–359. [CrossRef] [PubMed]
41. Nguyen, T.T.U.; Kim, H.W.; Kim, W. Effects of Probiotics, Prebiotics, and Synbiotics on Uremic Toxins, Inflammation, and Oxidative Stress in Hemodialysis Patients: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *J. Clin. Med.* **2021**, *10*, 4456. [CrossRef] [PubMed]
42. Wang, F.; Jiang, H.; Shi, K.; Ren, Y.; Zhang, P.; Cheng, S. Gut bacterial translocation is associated with microinflammation in end-stage renal disease patients. *Nephrology* **2012**, *17*, 733–738. [CrossRef] [PubMed]
43. Sun, J.; Axelsson, J.; Machowska, A.; Heimbürger, O.; Bárány, P.; Lindholm, B.; Lindström, K.; Stenvinkel, P.; Qureshi, A.R. Biomarkers of Cardiovascular Disease and Mortality Risk in Patients with Advanced CKD. *Clin. J. Am. Soc. Nephrol.* **2016**, *11*, 1163–1172. [CrossRef] [PubMed]
44. Rossi, M.; Campbell, K.L.; Johnson, D.W.; Stanton, T.; Vesey, D.A.; Coombes, J.S.; Weston, K.S.; Hawley, C.M.; McWhinney, B.C.; Ungerer, J.P.; et al. Protein-bound uremic toxins, inflammation and oxidative stress: A cross-sectional study in stage 3-4 chronic kidney disease. *Arch. Med. Res.* **2014**, *45*, 309–317. [CrossRef]
45. Inal, M.; Kanbak, G.; Sen, S.; Akyüz, F.; Sunal, E. Antioxidant status and lipid peroxidation in hemodialysis patients undergoing erythropoietin and erythropoietin-vitamin E combined therapy. *Free Radic Res.* **1999**, *31*, 211–216. [CrossRef]
46. Locatelli, F.; Canaud, B.; Eckardt, K.; Stenvinkel, P.; Wanner, C.; Zoccali, C. Oxidative stress in end-stage renal disease: An emerging threat to patient outcome. *Nephrol. Dial. Transplant.* **2003**, *18*, 1272–1280. [CrossRef]
47. El Mesallamy, F.A.F.; Elhefnawy, K.A.N.; El Said, H.H. Plasma Retinol and Malondialdehyde Levels among Hemodialysis Patients. *IJSR* **2015**, *4*, 193–200.
48. Morena, M.; Delbosc, S.; Dupuy, A.M.; Canaud, B.; Cristol, J.P. Overproduction of reactive oxygen species in end-stage renal disease patients: A potential component of hemodialysis-associated inflammation. *Hemodial. Int.* **2005**, *9*, 37–46. [CrossRef]
49. Atamer, A.; Kocyigit, Y.; A Ecder, S.; Selek, S.; Ilhan, N.; Ecder, T.; Atamer, Y. Effect of oxidative stress on antioxidant enzyme activities, homocysteine and lipoproteins in chronic kidney disease. *J. Nephrol.* **2008**, *21*, 924–930.
50. Yilmaz, M.I.; Saglam, M.; Caglar, K.; Cakir, E.; Sonmez, A.; Ozgurtas, T.; Aydin, A.; Eyileten, T.; Ozcan, O.; Acikel, C.; et al. The determinants of endothelial dysfunction in CKD: Oxidative stress and asymmetric dimethylarginine. *Am. J. Kidney Dis.* **2006**, *47*, 42–50. [CrossRef]
51. Xu, G.; Luo, K.; Liu, H.; Huang, T.; Fang, X.; Tu, W. The progress of inflammation and oxidative stress in patients with chronic kidney disease. *Ren. Fail.* **2015**, *37*, 45–49. [CrossRef] [PubMed]

52. Hatakka, K.; Martio, J.; Korpela, M.; Herranen, M.; Poussa, T.; Laasanen, T.; Saxelin, M.; Vapaatalo, H.; Moilanen, E.; Korpela, R. Effects of probiotic therapy on the activity and activation of mild rheumatoid arthritis—A pilot study. *Scand. J. Rheumatol.* **2003**, *32*, 211–215. [CrossRef] [PubMed]
53. Lamprecht, M.; Bogner, S.; Schippinger, G.; Steinbauer, K.; Fankhauser, F.; Hallstroem, S.; Schuetz, B.; Greilberger, J.F. Probiotic supplementation affects markers of intestinal barrier, oxidation, and inflammation in trained men. *Ann. Nutr. Metabol.* **2012**, *61*, 329.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.