

**ORAL SUPPLEMENTATION WITH PROBIOTIC  
LACTOBACILLUS REUTERI DSM 17938 AND VITAMIN D  
IMPROVES GASTROINTESTINAL SYMPTOMS AND  
INCREASES MEAN CIRCULATING 25- HYDROXYVITAMIN D  
IN COVID-19**

Journal: Minerva Gastroenterologica e Dietologica (New title: Minerva Gastroenterology)

Paper code: Minerva Gastroenterol-2949

Submission date: May 20, 2021

Article type: Original Article

Files:

1. Manuscript

Version: 1

Description: ORIGINAL MANUSCRIPT

File format: application/vnd.openxmlformats-officedocument.wordprocessingml.document

## MINERVA GASTROENTEROLOGY

A Journal on Gastroenterology, Nutrition and Dietetics

ORAL SUPPLEMENTATION WITH PROBIOTIC LACTOBACILLUS REUTERI DSM 17938 AND VITAMIND IMPROVES GASTROINTESTINAL SYMPTOMS AND INCREASES MEAN CIRCULATING 25- HYDROXYVITAMIN D IN COVID-19

Authors:

<sup>1</sup>Antonio Mastroianni, M.D.

<sup>1</sup>Valeria Vangeli, M.D.

<sup>1</sup>Luciana Chidichimo, M.D.

<sup>2</sup>Filippo Urso, M.D.

<sup>2</sup>Rossana Ritacca, M.D.

<sup>2</sup>Giuseppe De Marco, M.D.

<sup>3</sup>Francesca Greco, M.D.

<sup>3</sup>Daniela Perugini, M.D.

<sup>3</sup>Maria Vittoria Mauro, M.D.

<sup>1</sup>Sonia, Greco, M.D.

<sup>1</sup>Infectious & Tropical Diseases Unit,

<sup>2</sup>Hospital Pharmacy

<sup>3</sup>Microbiology & Virology

“Annunziata” Hub Hospital

Azienda Ospedaliera di Cosenza,

Cosenza, Cosenza, Italy

Correspondence Author:

Dr. Antonio Mastroianni

Infectious & Tropical Diseases Unit,

“Annunziata” Hub Hospital

Azienda Ospedaliera di Cosenza,

Viale della Repubblica s.n.c.

87100 Cosenza, Italy

Mobile & WA: +39 349 – 54.44.330

e-mail: [antoniomastroianni@yahoo.it](mailto:antoniomastroianni@yahoo.it)

Keyword: COVID-19, vitamin D, Lactobacillus reuteri, probiotics

Compliance with ethical standards

Conflict of interest: The authors declare no competing interests.

Author Contributions:

Mastroianni A: study design and original draft preparation;

Greco S, Chidichimo L, Mauro M.V., Vangeli V: study identification and data extraction and data interpretation;

All authors revised and approved the final version of the manuscript.

Acknowledgements: The authors are thankful to all the nursing staff for the magnificent work done in the health and human care of patients affected by Covid-19

## Abstract

**Background:** According to recent researches, gastrointestinal (GI) symptoms and vitamin D deficiency have been considered emerging features of hospitalized Covid-19 patients. There is a lack of studies evaluating the anti-inflammatory effect of probiotics combination with vitamin D, associated with their potential in gut microbiota modulation as an adjuvant treatment for Covid-19.

**Objectives:** The aims of this retrospective analysis were to assess the effectiveness of the effects of the probiotic *L.reuteri* DSM 17938 combined with vitamin D3 (Reuterin® D3 800 tablet) in the treatment of GI symptoms and on vitamin D deficiency in hospitalized Covid-19 patients.

**Methods:** We performed a retrospective, observational, case-control analysis to evaluate the safety and efficacy of the probiotic *L.reuteri* DSM 17938 combined with vitamin D3 (Reuterin® D3 800 tablet), on vitamin D deficiency and diarrhea duration and the frequency of new cases of otherwise unexplained cases of diarrhea (i.e. negative laboratory stool tests for infectious agents) in Covid-19 patients admitted at our ward, between March 6 2020 to May 6, 2021.

**Results:** Vitamin D3 and probiotic co-supplementation was associated with a significant improvement in different inflammatory biomarkers and serum levels of vitamin D, compared with the control-group ( $P = 0.001$ ). In patients treated with Reuterin® D3 800 tablet there was evidence of a lower frequency of admission to the ICU and a slightly lower mortality. Patient's evolution was marked by a clinical recovery, defined as an improvement of 1 point or more on the WHO Clinical Progression Scale in comparison to patients in the control group. The new occurrences of diarrhea were higher in the control group, regardless of the definition used. No treatment-emergent adverse events (AEs) were observed.

**Conclusions:** The results of our retrospective analysis suggest that a combined supplementation with vitamin D3 and the probiotic *L. reuteri* DSM 17938 (Reuterin® D3 800 tablet) may be a novel adjunctive insight in the management of Covid-19 patients, although future more large and high-quality studies are needed to confirm these findings, along with a state-of-the art analyses of the gut microbiota.

## Introduction

According to recent researches gastrointestinal symptoms and vitamin D deficiency have been considered emerging features of hospitalized Covid-19 patients.

There is a lack of studies evaluating the anti-inflammatory effect of probiotics combination with vitamin D, associated with their potential in gut microbiota modulation as an adjuvant treatment for Covid-19. The aims of these analyses were to assess the effectiveness of the effects of the probiotic *L.reuteri* DSM 17938 combined with vitamin D3 in the treatment of gastrointestinal (GI) symptoms and on vitamin D deficiency in hospitalized Covid-19 patients. Emerging evidence has indicated that gastrointestinal manifestations may play an important role in coronavirus disease 2019 (COVID-19). In one of the earliest studies of U.S. patients with Covid-19, researchers found one-third of patients reported symptoms affecting the digestive system, such as loss of appetite, nausea and diarrhea [1].

A recent review documented that, out of 1992 patients hospitalized in 36 centers, 53% of patients experienced at least 1 gastrointestinal symptom at any time during their illness, most commonly diarrhea (34%), nausea (27%), vomiting (16%), and abdominal pain (11%) [2].

A recent meta-analysis also suggests an increasing evidence of an association between GI symptoms and COVID-19 patients, with an increased risk of disease severity and negative complications, including acute respiratory distress syndrome, acute cardiac injury, and acute kidney injury, intensive care unit (ICU) admission, and mortality [3].

Vitamin D deficiency has been associated with increased inflammation and dysregulation of the immune system. Non-optimal vitamin D levels in adults and children in the world represent an increasing health problem, in particular among those subjects with Covid-19.

25-hydroxyvitamin D [25(OH)D] levels are lower in hospitalized COVID-19 patients than in population-based controls and are inversely associated with some inflammatory parameters, such as ferritin and D-dimer [4]. Vitamin D deficiency is associated with compromised inflammatory responses and higher pulmonary involvement in COVID-19 affected patients [5]. It has been suggested that vitamin D treatment could be useful for the prevention and treatment of COVID-19, in order to improve the immune balance and prevent the hyperinflammatory cytokine storm in all patients [5]. We retrospectively assessed the effectiveness of the effects of the probiotic *L.reuteri* DSM 17938 combined with vitamin D3 in the treatment of gastrointestinal (GI) symptoms and on vitamin D deficiency in hospitalized Covid-19 patients.

This novel combination of vitamin D3 and probiotic was used as an adjunct therapy to the standard of care (SOC) treatment recommended by the COVID-19 Treatment Guidelines Panel of the National Institutes of Health (<https://www.covid19treatmentguidelines.nih.gov/therapeutic->

1 [management](#) ), and by European Centre for Disease Prevention and Control  
2  
3 (<https://www.ecdc.europa.eu/en/covid-19/latest-evidence/treatment> ).

#### 4 **Methods**

5  
6 We performed a retrospective, observational, case–control analysis to evaluate the efficacy of  
7  
8 vitamin D3 and the probiotic *L. reuteri* DSM 17938 (Reuterin® D3 800 tablet) on vitamin D  
9  
10 deficiency and on stool output reduction, diarrhea duration and the frequency of new cases of  
11  
12 otherwise unexplained cases of diarrhea (i.e. negative laboratory stool tests for infectious agents)  
13  
14 and safety in Covid-19 patients admitted at our ward. This single-centered, retrospective,  
15  
16 observational study, was conducted at the Infectious & Tropical Diseases Unit of “Annunziata”  
17  
18 Hospital in Cosenza, Italy. The Institutional Review Board approved this case series using data  
19  
20 collected for routine clinical practice. Patients have given their informed consent for participation in  
21  
22 the research study. We analyzed 84 patients with confirmed COVID-19 hospitalized from March 6  
23  
24 2020 to May 6, 2021. SARS-CoV-2 was detected by nasopharyngeal swab polymerase chain  
25  
26 reaction (PCR) assay. Patients were provided with Reuterin® D3 800 tablet, a patented orange  
27  
28 flavored food supplement containing the probiotic strain *L. reuteri* DSM 17938 (108 CFU) and  
29  
30 Vitamin D3 (800UI), 2 tablets once a day during meals. SOC treatment included steroids,  
31  
32 anticoagulation, and antibiotics.

33  
34 The primary outcome measures was diarrhea, defined according to one of three definitions (i) three  
35  
36 or more loose or watery stools per day for  $\geq 48$  h; (ii) three or more loose or watery stools per day  
37  
38 for  $\geq 24$  h; or (iii) two or more loose or watery stools per day for  $\geq 24$  h. Secondary outcomes  
39  
40 included mortality during hospitalization; the number of patients admitted to the intensive care unit  
41  
42 (ICU); serum levels of 25OHD, and serum levels of different biomarkers of inflammation (C  
43  
44 reactive protein -CRP, Procalcitonin PCT), cell damage (hypersensitive troponin I, creatine kinase,  
45  
46 lactate dehydrogenase, ferritin, haptoglobin) and coagulation (fibrinogen, D-dimer, factor VIII ° and  
47  
48 acpr), interleukin 6 and lymphocytes count, dosed at the time of hospital admission.  
49  
50

#### 51 **Results**

52  
53 Patients took 2 tablets per day of Reuterin® D3 800, for an average of 20 (median, 18-48 days)  
54  
55 days. In all patients treated with Reuterin® D3 800 there was an improvement in the serum level of  
56  
57 25(OH)D after 3 weeks of treatment (Primary outcome measure,  $P=0.003$ ), while in patients in the  
58  
59 control the serum level of 25(OH)D did not change. All patients with GI symptoms at baseline  
60  
61 (70%) showed an improvement in digestive symptoms already after 7-14 days of treatment.  
62  
63 Negative clinical evolution was faster in patients with lower baseline 25(OH)D levels in the control  
64  
65 group. Vitamin D3 and probiotic co-supplementation was associated with a significant  
66  
67 improvement in CRP, ferritin, haptoglobin and LDH levels ( $P = 0.001$ ) compared with the control-

1 group. In patients treated with Reuterin D3 there was evidence of a lower frequency of admission to  
2 the ICU and a slightly lower mortality. Patient's evolution was marked by a clinical recovery,  
3 defined as an improvement of 1 point or more on the WHO Clinical Progression Scale in  
4 comparison to patients in the control group. No substantial difference were observed in terms of  
5 hospital stay. The new occurrences of diarrhea were higher in the control group, regardless of the  
6 definition used. No treatment-emergent adverse events (AEs) were observed.  
7  
8  
9

## 10 Discussion

11 Vitamin D deficiency has been associated with an increase in inflammatory cytokines, a  
12 significantly increased risk of severe pneumonia and thrombotic episodes, which are frequently  
13 observed in COVID-19. Some people with Covid-19 may develop GI such as diarrhea, loss of  
14 appetite, or vomiting. In view of the emerging evidence of a synergic health effects of co-  
15 supplementation with vitamin D and probiotics in modulating the gut microbiota and metabolome,  
16 with a dose-dependent improvement in health outcomes in various populations [6], we  
17 retrospectively evaluated whether a new food supplement containing *L. reuteri* DSM 17938 and  
18 vitamin D3 (Reuterin® D3) may improve GI symptoms and serum 25(OH)D in patients with  
19 moderate to severe Covid-19. Our findings suggested the efficacy of *L. reuteri* DSM 17938 and  
20 vitamin D3 in the new occurrence of diarrhea and for treatment of Covid-19-associated diarrhea,  
21 probably restoring dysbiosis in gut microbiota. Reuterin® D3 was well tolerated and no major side  
22 effects were found. Patients' satisfaction for therapy resulted higher in the treated group than in the  
23 control group. To our knowledge, this is the first report of increased circulating 25-hydroxyvitamin  
24 D in response to oral probiotic supplementation in patients with Covid-19. *L. reuteri* DSM 17938 is  
25 able to maintain the equilibrium of intestinal microflora, while vitamin D3 is useful to ensure the  
26 normal functioning of the immune system in patients with Covid-19. Patients who received a  
27 combination of vitamin D3 and probiotic supplementation achieved normalization of 25(OH)D  
28 serum levels, an improvement in GI symptoms and an improvement in inflammatory markers. *L.*  
29 *reuteri* DSM 17938 is a Gram-positive bacterium that naturally inhabits the gut of mammals. *L.*  
30 *reuteri* DSM 17938 is able to colonize the gut and it is useful to maintain the equilibrium of  
31 intestinal microflora. Vitamin D3 is useful to ensure the normal functioning of the immune system.  
32 Recent findings suggested that DSM 17938 is a potential probiotic for the prevention or treatment  
33 of liver failure [7]. Probiotic *L. reuteri* changed gut microbiota to modulate immune responses in  
34 murine experimental autoimmune encephalomyelitis [8].  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55

1 The mechanisms underlying the efficacy of *L.reuteri* seems to be the modulation of the intestinal  
2 microbiome with strengthening of the intestinal barrier, the ability to compete with microorganisms  
3 and the interaction with the innate and adaptive immune system associated.  
4

5  
6 In addition, there is emerging evidence that the vitamin D pathway might be important in gut  
7 homeostasis and in signaling between the microbiota and the host.  
8

9 Several lines of evidence suggest both an involvement of the GI tract in patients with Covid-19 and  
10 the influence of the gut microbiota dysbiosis on host inflammatory response in COVID-19 patients,  
11 through a modification of the plasma concentration of various cytokines that may persist even after  
12 the elimination of the virus [9], so the gut-brain axis can influence the immune response and the  
13 evolution of health status in patients with Covid-19 [10]. Some authors believe that the  
14 administration of probiotics can improve host immunity and alleviate gastrointestinal symptoms due  
15 to Covid-19 associated intestinal dysbiosis, representing very promising adjuvant immunobiotic  
16 neutraceutical agents in patients with Covid-19 [11]. Din AU et al. [12] recently suggested that  
17 probiotics could mitigate the severity of the disease enhancing epithelial barrier function, as anti-  
18 inflammatory, improving gut microbial diversity, as the antagonist, for various harmful bacterial  
19 strains in the gut, blocking or enhancing multiple signalling pathways [12]. We hypothesized that  
20 better understanding of the gut microbiome in Covid-19 patients could be useful for improving  
21 health in these patients. Probiotics can inhibit the growth of pathogens and improve the  
22 immunology and metabolic functions of the host. The exact mechanism involved in this process in  
23 Covid-19 patients could be better explored based on the integration of the cytokine matrix, gut  
24 microbiota and metabolome data. Strategies to restore dysfunctional vitamin D receptor (VDR)  
25 expression in inflamed mucosa, might alleviate biomarkers of inflammation and oxidative stress in  
26 patients with Covid-19, enhancing the host's protection against inflammation and infection.  
27

28 The limitations of our analysis may be the small sample size, short duration of follow-up, and lack  
29 of state-of-the art analyses of the gut microbiota. One approach is to identify and treat vitamin D  
30 deficiency, especially in high-risk individuals such as the elderly, patients with comorbidities, and  
31 nursing home residents, who are the main target population for the COVID-19  
32

33 There is a lack of studies evaluating the anti-inflammatory effect of probiotics combination with  
34 vitamin D, associated with their potential in gut microbiota modulation as an adjuvant treatment for  
35 Covid-19. Further studies with more cases and with higher doses of the drug are necessary to  
36 confirm our results. In conclusion, we suggest that a combined supplementation with vitamin  
37 Reuterin® D3 800 tablet may be a novel adjunctive insight in the management of Covid-19  
38 patients, although future more large and high-quality studies are needed to confirm these findings.  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55

## References

- 1.Cholankeril G, Podboy A, Aivaliotis VI, et al. Association of Digestive Symptoms and Hospitalization in Patients With SARS-CoV-2 Infection. *Am J Gastroenterol*. 2020 Jun 10 : 10.14309/ajg.0000000000000712].
- 2.Elmunzer BJ, Spitze R L, Foster L, et al.. Digestive Manifestations in Patients Hospitalized With Coronavirus Disease 2019. *Clin Gastroent Hepatology*. 2020;S1542-3565(20)31371-9. Advance online publication. <https://doi.org/10.1016/j.cgh.2020.09.041>.
- 3.Elshazli RM, Kline A, Elgaml A, et al. Gastroenterology manifestations and COVID-19 outcomes: A meta-analysis of 25,252 cohorts among the first and second waves. *J Med Virol* 2021; 93: 2740 – 2768
- 4.José L Hernández, Daniel Nan, Marta Fernandez-Ayala, Mayte García-Unzueta, Miguel A Hernández-Hernández, Marcos López-Hoyos, Pedro Muñoz-Cacho, José M Olmos, Manuel Gutiérrez-Cuadra, Juan J Ruiz-Cubillán, Javier Crespo, Víctor M Martínez-Taboada, Vitamin D Status in Hospitalized Patients with SARS-CoV-2 Infection, *The Journal of Clinical Endocrinology & Metabolism*, Volume 106, Issue 3, March 2021, Pages e1343–e1353, <https://doi.org/10.1210/clinem/dgaa733> ).
- 5.Ricci, A., Pagliuca, A., D'Ascanio, M. et al. Circulating Vitamin D levels status and clinical prognostic indices in COVID-19 patients. *Respir Res* 22, 76 (2021). <https://doi.org/10.1186/s12931-021-01666-3>
- 6.Abboud M, Rizk R, AlAnouti F, et al. The Health Effects of Vitamin D and Probiotic Co-Supplementation: A Systematic Review of Randomized Controlled Trials. *Nutrients* 2021; 13:111. <https://doi.org/10.3390/nu13010111>



1 7.Jiang H, Yan R, Wang K, Wang Q, Chen X, Chen L, Li L, Lv L. Lactobacillus reuteri DSM  
2 17938 alleviates d-galactosamine-induced liver failure in rats. Biomed Pharmacother. 2021  
3 Jan;133:111000. doi: 10.1016/j.biopha.2020.111000.  
4

5  
6  
7 8.He B, Hoang TK, Tian X, Taylor CM, Blanchard E, Luo M, Bhattacharjee MB, Freeborn J, Park  
9 S, Couturier J, Lindsey JW, Tran DQ, Rhoads JM and Liu Y (2019) Lactobacillus reuteri Reduces  
10 the Severity of Experimental Autoimmune Encephalomyelitis in Mice by Modulating Gut  
11 Microbiota. Front. Immunol. 10:385. doi: 10.3389/fimmu.2019.00385  
12  
13

14  
15 9.Yeoh YK, Zuo T, Lui GC, et al Gut microbiota composition reflects disease severity and  
16 dysfunctional immune responses in patients with COVID-19. Gut 2021;70:698-7069  
17  
18

19  
20 10.Chhibber-Goel, J., Gopinathan, S. & Sharma, A. Interplay between severities of COVID-19 and  
21 the gut microbiome: implications of bacterial co-infections?. Gut Pathog 13, 14 (2021).  
22 <https://doi.org/10.1186/s13099-021-00407-7>  
23  
24

25  
26 11.Gohil K, Samson R, Dastager S, Dharne M. Probiotics in the prophylaxis of COVID-19:  
27 something is better than nothing. 3 Biotech. 2021 Jan;11(1):1. doi: 10.1007/s13205-020-02554-1.  
28 Epub 2020 Nov 26. PMID: 33262924; PMCID: PMC7690945  
29  
30

31  
32 12.Din AU, Mazhar M, Waseem M, et al. SARS-CoV-2 microbiome dysbiosis linked disorders and  
33 possible probiotics role. Biomed Pharmacother. 2021;133:110947.  
34 doi:10.1016/j.biopha.2020.110947  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55