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CLINICAL TRIAL STUDY

Vitamin Producing Lactic Acid Bacteria as Complementary Treatments for Intestinal Inflammation

Alejandra de Moreno de LeBlanc[1], Romina Levit[1], Graciela Savoy de Giori[1,2] and Jean Guy LeBlanc[1,*]

[1]Centro de Referencia para Lactobacilos (CERELA-CONICET). Chacabuco 145, (T4000ILC) San Miguel de Tucumán, Tucumán, Argentina; [2]Cátedra de Microbiología Superior, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, San Miguel de Tucumán, Tucumán, Argentina CERELA-CONICET, San Miguel de Tucumán, Tucumán, C.P. 4000, Argentina

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Abstract: ***Background:*** Current therapies for against inflammatory bowel disease (IBD) are sometimes limited by high costs, high toxicities and/or undesirable side effects, reasons for which new treatments are constantly being developed and studied. In this regards, an increasing mass of data has demonstrated that fecal transplantations and probiotic supplementations have shown promising effects and could be considered as adjunct IBD treatments to decrease some of the unwanted side effects caused by primary treatments. Furthermore, there is also mounting evidence that suggests that certain vitamins could provide anti-inflammatory effects and it has been shown that certain strains of lactic acid bacteria (LAB), the most commonly used probiotic microorganisms, can produce biologically active forms of certain vitamins.

Objective: To discuss the potential role of the vitamin-producing LAB on intestinal inflammatory diseases.

Method: A thorough search of bibliographic databases for peer-reviewed research on the effect of vitamins produced by LAB on inflammatory processes was performed.

Results: There is mounting research that vitamin producing LAB could provide anti-inflammatory effects.

Conclusion: The potential role of vitamin producing LAB was discussed not only because they could be used to decrease inflammation but also because they could provide the host with essential nutrients that are normally deficient in IBD patients due to altered intestinal morphologies.

Keywords: Colitis, folate, intestinal inflammation, lactic acid bacteria, mucositis, probiotics, riboflavin, vitamins.

1. INTRODUCTION

The pathogenesis of inflammatory bowel disease (IBD) is still unclear, but there are multifactorial factors that affect their development such as genetics, immunology, environmental and microbial factors. IBD has become global diseases with high incidence rates that affect the quality of life of patients and also increase their risk of colon cancer development [1]. Current therapies for against IBD are limited by high costs, high toxicities and undesirable side effects [2, 3]. This emphasizes the need to search for novel therapeutic options. In this regard, many groups have been studying ways of modulating the gut microbiota in

*Address correspondence to this author at the CERELA-CONICET, San Miguel de Tucumán, Tucumán, C.P. 4000, Argentina; Tel/Fax: +54-381-4310465;
E-mails: leblanc@cerela.org.ar, leblancjeanguy@gmail.com

order to prevent or treat IBD patients. The use of fecal transplantation and probiotics has been shown to be very promising in this regards. It is well documented that the microbiota and its modulation can affect many aspects of human health. In this Topic Review, the potential role of vitamin-producing lactic acid bacteria (LAB), the most commonly used probiotic microorganisms, on intestinal inflammatory diseases will be discussed.

In addition to being defined as essential to promote the growth and health of consumers, many vitamins are involved as co-factors in numerous metabolic reactions and their deficiency has been shown to cause many physiological manifestations beyond those traditional studied by a nutritionist.

It is well known that because of changes in eating habits such as malnutrition, a large proportion of the world's population does not consume the daily recommended intakes of vitamins as suggested by the WHO [4]. In order to combat this problem, many countries have adopted mandatory fortification of foods of mass consumption with specific vitamins and minerals; however, in most cases, chemical forms of vitamins (such as folic acid) are used and these do not exert the same physiological responses as the natural vitamins found in foods or produced by certain microorganisms (including LAB). Furthermore, patients that suffer from intestinal inflammatory disorders or that receive certain anti-inflammatory and chemotherapy drugs can have impaired vitamin intakes that could cause vitamin deficiencies and affect the outcome of their primary treatments. The use of lactic acid bacteria can be seen as a promising complementary treatment for such patients because i) it is well known that these bacteria are normally used as probiotics and that these beneficial microbes can positively affect IBD patients [5], ii) it has been shown that some strains of LAB can produce important amounts of vitamins that would be useful to prevent deficiencies, and iii) these vitamins can also produce beneficial effects because some can provide antioxidant effects or prevent inflammatory symptoms. The use of probiotics such as LAB to improve IBD patient's treatment outcomes due to their intrinsic immune-modulatory properties has been extensively reviewed [5] and will not be discussed further here.

1.1. Vitamin-producing LAB

There is an increase in studies that have shown the capacity of certain strains of the LAB to produce vitamins, especially those contained in the water-soluble B-group [6]. It has been shown that LAB can produce folates, riboflavina and even vitamin B12 and that this production is a strain-dependent trait [7]. Vitamin producing strains have been isolated from numerous ecological niches that include the human gastrointestinal tract, animal milks (including humans, dogs and horses), fermented foods (corn, potatoes, milks), flours and grains [8-14]. These vitamin-producing strains have traditionally been used for the development of novel biofortified foods that could be used as an alternative to fortification with chemically synthesized or purified derivatives of vitamins. In addition to show that LAB is able to increase vitamin concentrations during their fermentation process, it has been reported that these bio-enriched foods or vitamin-producing LAB can revert or prevent vitamin deficiencies in animal models [15-19].

Another use of vitamin-producing strains is as probiotics, where it is proposed that the beneficial microorganism could deliver or produce vitamins in the digestive tract. In this regard, many strains with well demonstrated probiotic properties have been studied and some have been identified as being able to biosynthesize vitamins de novo in the gastrointestinal tract due to the presence of all biosynthesis genes [20, 21]. Some authors have even proposed vitamin production as a probiotic trait [22].

Besides naturally occurring vitamin producing strains, it has been shown that genetically modifying LAB could increase vitamin production [23]. However, due to consumer concerns against the insertion of GM foods in the food chain, the use of these strains would be greatly limited. For this reason, many groups have been able to induce spontaneous mutations in vitamin-biosynthesis regulatory genes giving rise to riboflavin over-producing LAB that are not genetically modified and can successfully be used for the development of novel foods [24-27].

1.2. Vitamins and Inflammation

As stated earlier, it has been shown that certain vitamins can exert beneficial properties besides their nutritional value. In this section, a brief overview of the relationship vitamins and inflammatory processes will be discussed with special emphasis placed on B group vitamins that are produced by specific LAB strains.

Riboflavin (vitamin B2) plays an essential role in cellular metabolism, being the precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), co-enzymes required by all flavoproteins including glutathione reductase (GSH) which protects cells from the harmful effect of reactive oxygen species (ROS). The unbalanced production of ROS is commonly associated with chronic intestinal inflammation in the early stages of IBD, and their destructive effects on DNA, proteins and lipids may contribute to initiation and progression of IBD [28]. For this reason, foods rich in antioxidants or antioxidants administered as supplements have been used to alleviate ROS-induced damage. In this sense, the relationship between antioxidant vitamins and IBD was studied and it was shown that supplementation with vitamins E and C significantly reduced oxidative stress in patients with Crohn's disease (CD) [29-30]. Furthermore, preclinical and clinical data suggested that vitamin D has therapeutic potential in IBD, particularly in CD patients [31] and in those suffering from irritable bowel syndrome [32]. Patients with vitamin D deficiency displayed increased ulcerative colitis (UC) severity and a lower quality of life in a cohort of patients with IBD [33, 34].

Although riboflavin is clearly an anti-oxidant enzyme cofactor, little research has shown its use against IBD; however, there are some studies that have clearly shown that this vitamin can be involved in an anti-inflammatory process. Oral administration of riboflavin prevented carbon tetrachloride-induced liver damage in rats causing the normalization of serum hepatic enzymes and oxidant parameters in liver (including GSH activity) [35]. In this same work, it was also shown the riboflavin intake inhibited the release of the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α) from rat leukocytes. From a more mechanistic point of view, it was shown that short-term riboflavin deficiency significantly impairs the ability of macrophages to induce a proper immune response, while riboflavin enrichment decreases the pro-inflammatory activation of macrophages [36]. LPS-induced macrophages that were supplemented with riboflavin showed lower mortality accompanied by the higher Hsp72 expression, reduction of Toll-like receptor 4 (TLR4) and TNF-α, and elevation of NO, IL-6, and IL-10 compared to when riboflavin was removed from the culture media. It was also reported that riboflavin could alleviate oxidative injuries by scavenging the free radicals reducing early graft lipid peroxidation, leukocytic infiltration, and cytokine production [37]. Moreover, riboflavin supplementation reduced inflammation caused by oxidative stress during diabetes [38]. In addition to being involved in inflammation, it has also been shown that the presence of riboflavin is essential to avoid malformation of intestinal cells [39]. All these results clearly show that riboflavin has proven anti-inflammatory effects and should be evaluated in IBD patients.

Folates and folic acid (vitamin B9) also play an important role in cellular metabolic events. The anti-inflammatory properties of this vitamin have been reported in different pathologies. The administration of folic acid to patients suffering arterial hypertension has been associated with decreased concentrations of homocysteine in serum, and indirectly to decreases of other indicators of inflammation [40]. A trial with obese children and adolescents showed that the treatment with both metformin and folic acid has been associated to decreased insulin resistance and homocysteine levels and that the decrease of the pro-inflamma-tory cytokines TNF-α, IL-8 and IL-6 was related to folic acid administration [41]. Regarding IBD, it has been reported that these diseases can be associated with folate deficiency [42], and the supplementation with folic acid could reduce the risk to develop colorectal cancer in patients suffering IBD [43]. These results suggest a beneficial effect of folic acid against inflammation; however, no studies have been performed with natural folates that are found in foods or that are produced by certain strains of the LAB.

1.3. Vitamin-Producing LAB and Inflammation

Although it is clear that vitamin supplementation can be effective in IBD and other inflammatory diseases and that LAB can produce vitamins, little research has been performed that show an association between the vitamin-producing LAB and anti-inflammatory effects.

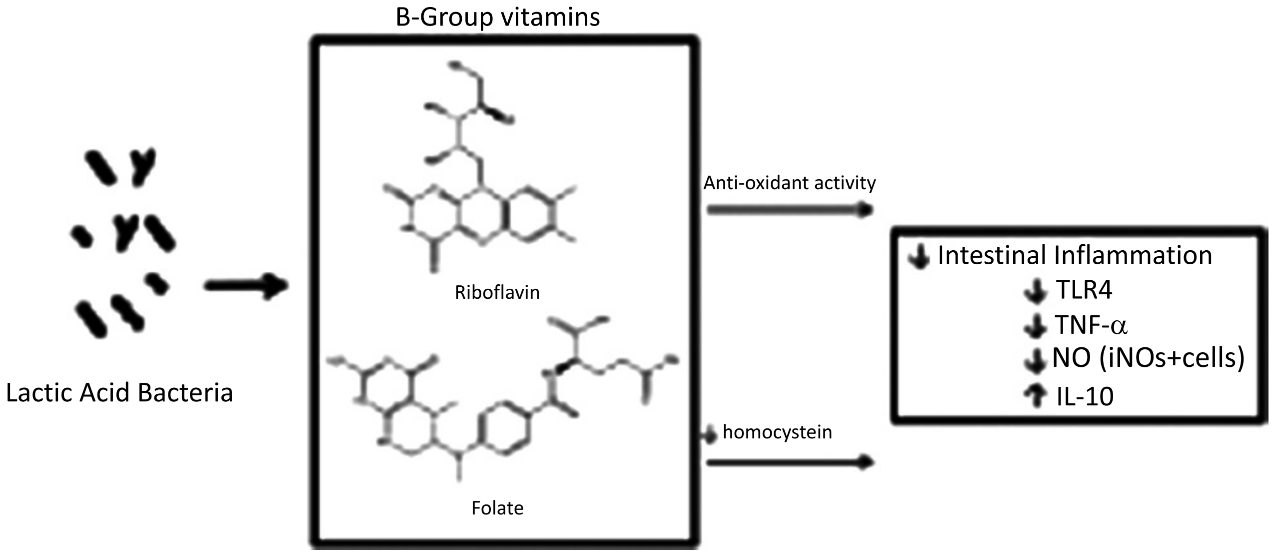

Fig. (1). Schematic representation of lactic acid bacteria that produce B-group vitamins (folate, riboflavin) that can induce a reduction of intestinal inflammation and decreasing pro-inflammatory regulators and increasing the anti-inflammatory cytokine IL-10.

Recently it was suggested that vitamin D and its receptor (VDR) can contribute to probiotic actions that are involved in regulation of innate immune functions *via* TLRs, NF-κB, MAPK and secretion of anti- and pro-inflammatory molecules [44]. However, probiotic microorganisms cannot synthesize this vitamin.

In a recent study, it was shown that the human-derived folate (vitamin B9) producing strain *Lactobacillus* (*L.*) *reuteri* ATCC PTA 6475 is able to suppress the production of the pro-inflammatory cytokine TNF-α, and are protective in a mouse model of colitis [45]. This strain is capable of producing 5,10-methenyltetrahydrofolic acid polyglutamates, a biologically active form of folate. It was shown that the folC2 gene, involved in this folate biosynthesis, was related with the suppression of TNF-α by activated human monocytes and with the anti-inflammatory effect of *L. reuteri* 6475 in a trinitrobenzene sulfonic acid (TNBS)-induced mouse model of acute colitis. The folC2 mutant yielded diminished *hdc* cluster expression, this latter gene being involved in immunomodulation by converting histidine to histamine. These results confirm that the oral administration of folate-producing probiotic strains may confer a more efficient protection against inflammation and cancer by exerting the beneficial effects of providing folate and by delivering it to colonic-rectal cells [46].

The most conclusive results that vitamin producing LAB strains could be used as an alternative treatment against IBD has been shown in two recently published studies using TNBS induced colitis animal models. In the first, it was described that TNBS-inoculated mice that received soymilk fermented by *L. Plantarum* CRL 2130, a riboflavin over-producing strain, showed a decrease in weight loss, lower damage scores in their large intestines, lower microbial translocation to liver and decreased pro-inflammatory cytokines levels in their intestinal fluids compared to animals that received unfermented soymilk or soymilk fermented by a non-riboflavin-producing *L. plantarum* strain [47]. This is the first report that demonstrates food fermented with a riboflavin-producing LAB was able to prevent experimental colitis in a murine model. These same authors then published a study where different riboflavin producing strains (not included in a food matrix) showed similar beneficial effects in the chemically induced colitis rodent model. In this latest study [48] it was shown that animals given either one of the four riboflavin-producing strains (*L. plantarum* CRL2130, L. paracasei CRL76, *L. bulgaricus* CRL871 or *Streptococcus thermophilus* CRL803) presented lower macroscopic and histologic damage scores, lower microbial translocation to liver, significant decreases of iNOs+ cells in their large intestines and decreased pro-inflammatory cytokines, compared to mice that received strains that did not produce this vitamin (Fig. **1**). These results clearly show that the administration of riboflavin-producing strains prevented the intestinal damage induced by TNBS in mice and that a riboflavin-producing phenotype in LAB represents a potent tool to select them for preventing/treating IBD.

Following these results, it was shown that the riboflavin overproducing strain *L. plantarum* CRL2130 significantly attenuated pathological changes of the intestines and increased pro-inflammatory cytokine profiles in animals with 5-fluorouracil-induced mucositis [49]. Using this same model, it was also shown that folate producing LAB were also able to decrease mucositis symptoms (unpublished data), suggesting that vitamin producing strains could, in fact, be useful as therapeutical agents against a wide range of pathologies that involve intestinal inflammation (Fig **1**).

CONCLUSION

The use of lactic acid bacteria can be seen as a promising complementary treatment for patients suffering from a wide range of inflammatory diseases, especially those of the gastrointestinal tract. Some of these beneficial bacteria have innate immune regulatory properties and it has been shown that some strains can produce important amounts of vitamins that would be useful to prevent deficiencies as well as provide antioxidant effects. The use of probiotics such as LAB to improve IBD patient's treatment outcomes due to their intrinsic immune-modulatory properties and vitamin production in the gastrointestinal tract still requires much study but is promising for the overall well-being of patients suffering IBD.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Chen, Y.; Friedman, M.; Liu, G.; Deodhar, A.; Chu, C. Q. Do tumor necrosis factor inhibitors increase cancer risk in patients with chronic immune-mediated inflammatory disorders? *Cytokine,* **2018**, *101*, 78-88.

[2] Araujo, D. F. S.; Guerra, G. C. B.; Junior, R. F. A.; Antunes de Araujo, A.; Antonino de Assis, P. O.; Nunes de Medeiros, A.; Formiga de Sousa, Y. R.; Pintado, M. M. E.; Galvez, J.; Queiroga, R. Goat whey ameliorates intestinal inflammation on acetic acid-induced colitis in rats. *J. Dairy Sci.,* **2016**, *99*(12), 9383-9394.

[3] Zhang, W.; Teng, G.; Wu, T.; Tian, Y.; Wang, H. Expression and clinical significance of elafin in inflammatory bowel disease. *Inflamm. Bowel Dis.,* **2017**, *23*(12), 2134-2141.

[4] FAO/WHO, Human vitamin and mineral requirements. In *Human vitamin and mineral requirements*, FAO/WHO ed.; Bangkok, Thailand, **2002**.

[5] LeBlanc, J. G.; de Moreno de LeBlanc, A. Probiotics in inflammatory bowel diseases and cancer prevention. In *Bioactive Foods in Promoting Health: Probiotics, Prebiotics, and Synbiotics* 2nd ed.; Watson, R. R.; Preedy, V. R., Eds. Academic Press (Elsevier): Oxford, UK, **2015**, 755-771.

[6] LeBlanc; Laino, J. E.; Juarez del Valle, M.; Vannini, V.; van Sinderen, D.; Taranto, M. P.; Font de Valdez, G.; Savoy de Giori, G.; Sesma, F. B-Group vitamin production by lactic acid bacteria - current knowledge and potential applications. *J. Appl. Microbiol.,* **2011**, *111*(6), 1297-1309.

[7] LeBlanc, J. G.; Laiño, J. E.; Juarez del Valle, M.; Savoy de Giori, G.; Sesma, F.; Taranto, M. P. B-Group Vitamins Production by Probiotic Lactic Acid Bacteria. In *Biotechnology of Lactic Acid Bacteria: Novel Applications*, Second ed.; Mozzi, F.; Raya, R. R.; Vignolo, G. M., Eds. Wiley Blackwell: Ames, IA, USA, **2015**, pp. 279-296.

[8] Cardenas, N.; Laino, J. E.; Delgado, S.; Jimenez, E.; Juarez del Valle, M.; Savoy de Giori, G.; Sesma, F.;

Mayo, B.; Fernandez, L.; LeBlanc, J. G.; Rodriguez, J. M. Relationships between the genome and some phenotypical properties of Lactobacillus fermentum CECT 5716, a probiotic strain isolated from human milk. *Appl. Microbiol. Biotechnol.,* **2015**, *99*(10), 4343-4353.

[9] Carrizo, L. S.; Montes de Oca, C.; Laiño, J. E.; Suarez, N. E.; Vignolo, G.; LeBlanc, J. G.; Rollan, G. Ancestral andean grain quinoa as a source of functional lactic acid bacteria. *Food Res. Int.,* **2016**, *89*, 488-494.

[10] Laino, J. E.; LeBlanc, J. G.; Savoy de Giori, G. Production of natural folates by lactic acid bacteria starter cultures isolated from artisanal Argentinean yogurts. *Canadian J. Microbiol.,* **2012**, *58*(5), 581-588.

[11] Salvucci, E.; LeBlanc, J. G.; Pérez, G. Technological properties of Lactic acid bacteria isolated from raw cereal material. *LWT-Food Sci. Technol.,* **2016**, *70*, 185-191.

[12] Carrizo, S. L.; Montes de Oca, C. E.; Hebert, M. E.; Saavedra, L.; Vignolo, G.; LeBlanc, J. G.; Rollan, G. C. Lactic acid bacteria from andean grain amaranth: a source of vitamins and functional value enzymes. *J. Mol. Microbiol. Biotechnol*., **2017**, *27*(5), 289-298.

[13] Jiménez, E.; Yépez, A.; Pérez-Cataluña, A.; Vásquez, E. R.; Dávila, D. Z.; Vignolo, G.; Aznar, R. Exploring diversity and biotechnological potential of lactic acid bacteria from tocosh-traditional Peruvian fermented potatoes-by high throughput sequencing (HTS) and culturing. *LWT-Food Sci. Technol.,* **2018**, *87*, 567-574.

[14] Russo, P.; de Chiara, M. L. V.; Capozzi, V.; Arena, M. P.; Amodio, M. L.; Rascón, A.; Dueñas, M. T.; López, P.; Spano, G. Lactobacillus plantarum strains for multifunctional oat-based foods. *LWT-Food Sci. Technol.,* **2016**, *68*, 288-294.

[15] Juarez del Valle, M.; Laiño, J. E.; de Moreno de LeBlanc, A.; Savoy de Giori, G.; LeBlanc, J. G. Soymilk fermented with a riboflavin producing Lactobacillus plantarum CRL 2130 reverts and prevents ariboflavinosis in murine models. *Br. J. Nutri.*, **2016**, *116*, 1229-1235.

[16] Laiño, J. E.; Zelaya, H.; Juarez del Valle, M.; Savoy de Giori, G.; LeBlanc, J. G. Milk fermented with selected strains of lactic acid bacteria is able to improve folate status of deficient rodents and also prevent folate deficiency. *J. Functional Foods,* **2015**, *17*, 22-32.

[17] LeBlanc, J. G.; Burgess, C.; Sesma, F.; Savoy de Giori, G.; van Sinderen, D. Ingestion of milk fermented by genetically modified *Lactococcus lactis* improves the riboflavin status of deficient rats. *J. Dairy. Sci.*, **2005**, *88*(10), 3435-3442.

[18] LeBlanc, J. G.; Rutten, G.; Bruinenberg, P.; Sesma, F.; de Giori, G. S.; Smid, E. J. A novel dairy product fermented with Propionibacterium freudenreichii improves the riboflavin status of deficient rats. *Nutrition,* **2006**, *22*(6), 645-651.

[19] LeBlanc, J. G.; Sybesma, W.; Starrenburg, M.; Sesma, F.; de Vos, W. M.; de Giori, G. S.; Hugenholtz, J. Supplementation with engineered Lactococcus lactis improves the folate status in deficient rats. *Nutrition*, **2010**, *26*(7-8), 835-841.

[20] LeBlanc, J. G.; Milani, C.; de Giori, G. S.; Sesma, F.; van Sinderen, D.; Ventura, M. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr. Opin. Biotechnol.,* **2013**, *24*(2), 160-168.

[21] LeBlanc, J. G.; Chain, F.; Martin, R.; Bermudez-Humaran, L. G.; Courau, S.; Langella, P., Beneficial effects on host energy metabolism of short-chain fatty acids and vitamins produced by commensal and probiotic bacteria. *Microb. Cell Fact.,* **2017**, *16*(1), 79.

[22] Rossi, M.; Amaretti, A.; Raimondi, S. Folate production by probiotic bacteria. *Nutrients,* **2011**, *3*(1), 118-134.

[23] Sybesma, W.; Burgess, C.; Starrenburg, M.; van Sinderen, D.; Hugenholtz, J. Multivitamin production in Lactococcus lactis using metabolic engineering. *Metab. Eng.,* **2004**, *6*(2), 109-115.

[24] Burgess, C. M.; O'Connell-Motherway, M.; Sybesma, W.; Hugenholtz, J.; van Sinderen, D. Riboflavin production in *Lactococcus lactis*: potential for *in situ* production of vitamin-enriched foods. *Appl. Environ. Microbiol.,* **2004**, *70*(10), 5769-5777.

[25] Capozzi, V.; Menga, V.; Digesu, A. M.; De Vita, P.; van Sinderen, D.; Cattivelli, L.; Fares, C.; Spano, G. Biotechnological production of vitamin B2-enriched bread and pasta. *J. Agric. Food Chem*., **2011**, *59*(14), 8013-8020.

[26] Russo, P.; Capozzi, V.; Arena, M. P.; Spadaccino, G.; Duenas, M. T.; Lopez, P.; Fiocco, D.; Spano, G. Riboflavin-overproducing strains of Lactobacillus fermentum for riboflavin-enriched bread. *Appl. Microbiol. Biotechnol.,* **2014**, *98*(8), 3691-3700.

[27] Juarez del Valle, M.; Laiño, J. E.; Savoy de Giori, G.; LeBlanc, J. G. Riboflavin producing lactic acid bacteria as a biotechnological strategy to obtain bio-enriched soymilk. *Food Res. Int.,* **2014**, *62*, 1015-1019.

[28] Moura, F. A.; de Andrade, K. Q.; Dos Santos, J. C.; Araujo, O. R.; Goulart, M. O. Antioxidant therapy for treatment of inflammatory bowel disease: Does it work? *Redox Biol.,* **2015**, *6*, 617-639.

[29] Aghdassi, E.; Wendland, B. E.; Steinhart, A. H.; Wolman, S. L.; Jeejeebhoy, K.; Allard, J. P. Antioxidant vitamin supplementation in Crohn's disease decreases oxidative stress: a randomized controlled trial. *Am. J. Gastroenterol.,* **2003**, *98*(2), 348-353.

[30] Roggenbuck, C.; Lammert, F.; Berthold, H. K.; Giese, T.; Stallmach, A.; Stehle, P.; Ellinger, S. High-dose oral supplementation of antioxidants and glutamine improves the antioxidant status in patients with Crohn's disease: A pilot study. *e-SPEN,* **2008**, *3*(5), e246-e253.

[31] Hlavaty, T.; Krajcovicova, A.; Payer, J. Vitamin D therapy in inflammatory bowel diseases: who, in what form, and how much? *J. Crohns Colitis,* **2015**, *9*(2), 198-209.

[32] Tazzyman, S.; Richards, N.; Trueman, A. R.; Evans, A. L.; Grant, V. A.; Garaiova, I.; Plummer, S. F.; Williams, E. A.; Corfe, B. M. Vitamin D associates

with improved quality of life in participants with irritable bowel syndrome: outcomes from a pilot trial. *BMJ Open Gastroenterol*., **2015**, *2*(1), e000052.

[33] Blanck, S.; Aberra, F. Vitamin d deficiency is associated with ulcerative colitis disease activity. *Dig. Dis.. Sci.,* **2013**, *58*(6), 1698-1702.

[34] Castro, F. D.; Magalhaes, J.; Carvalho, P. B.; Moreira, M. J.; Mota, P.; Cotter, J. Lower levels of vitamin d correlate with clinical disease activity and quality of life in inflammatory bowel disease. *Arquivos de gastroenterol.,* **2015**, *52*(4), 260-265.

[35] Al-Harbi, N. O.; Imam, F.; Nadeem, A.; Al-Harbi, M. M.; Iqbal, M.; Ahmad, S. F. Carbon tetrachloride-induced hepatotoxicity in rat is reversed by treatment with riboflavin. *Int. Immunopharmacol.,* **2014**, *21*(2), 383-388.

[36] Mazur-Bialy, A. I.; Pochec, E.; Plytycz, B. Immunomodulatory effect of riboflavin deficiency and enrichment-reversible pathological response versus silencing of inflammatory activation. *J. physiol. Pharmacol.*, **2015**, *66*(6), 793-802.

[37] Iwanaga, K.; Hasegawa, T.; Hultquist, D. E.; Harada, H.; Yoshikawa, Y.; Yanamadala, S.; Liao, H.; Visovatti, S. H.; Pinsky, D. J. Riboflavin-mediated reduction of oxidant injury, rejection, and vasculopathy after cardiac allotransplantation. *Transplantation,* **2007**, *83*(6), 747-753.

[38] Alam, M. M.; Iqbal, S.; Naseem, I. Ameliorative effect of riboflavin on hyperglycemia, oxidative stress and DNA damage in type-2 diabetic mice: Mechanistic and therapeutic strategies. *Arch. Biochem. Biophys.,* **2015**, *584*, 10-19.

[39] Powers, H. J. Riboflavin (vitamin B-2) and health. *Am. J. Clin. Nutr.,* **2003**, *77*(6), 1352-1360.

[40] Baszczuk, A.; Thielemann, A.; Musialik, K.; Kopczynski, J.; Bielawska, L.; Dzumak, A.; Kopczynski, Z.; Wysocka, E. The impact of supplementation with folic acid on homocysteine concentration and selected lipoprotein parameters in patients with primary hypertension. *J. Nutr. Sci. Vitaminol., (Tokyo)* **2017**, *63*(2), 96-103.

[41] Dehkordi, E. H.; Sattari, F.; Khoshdel, A.; Kasiri, K. Effect of folic acid and metformin on insulin resistance and inflammatory factors of obese children and adolescents. *J. Res. Med. Sci.,* **2016**, *21*, 71.

[42] Pan, Y.; Liu, Y.; Guo, H.; Jabir, M. S.; Liu, X.; Cui, W.; Li, D. Associations between folate and vitamin B12 levels and inflammatory bowel disease: A meta-analysis. *Nutrients,* **2017**, *9*(4), 382.

[43] Burr, N. E.; Hull, M. A.; Subramanian, V. Folic acid supplementation may reduce colorectal cancer risk in patients with inflammatory bowel disease: A systematic review and meta-analysis. *J. Clin. Gastroenterol.,* **2017**, *51*(3), 247-253.

[44] Shang, M.; Sun, J. Vitamin D/VDR, Probiotics, and Gastrointestinal Diseases. *Curr. Med. Chem.,* **2017**, *24*(9), 876-887.

[45] Thomas, C. M.; Saulnier, D. M.; Spinler, J. K.; Hemarajata, P.; Gao, C.; Jones, S. E.; Grimm, A.; Balderas, M. A.; Burstein, M. D.; Morra, C.; Roeth, D.; Kalkum, M.; Versalovic, J. FolC2-mediated folate metabolism contributes to suppression of inflammation by probiotic Lactobacillus reuteri. *Microbiol. Open,* **2016**, *5*(5), 802-818.

[46] Pompei, A.; Cordisco, L.; Amaretti, A.; Zanoni, S.; Matteuzzi, D.; Rossi, M. Folate production by bifidobacteria as a potential probiotic property. *Appl. Environ. Microbiol.,* **2007**, *73*(1), 179-185.

[47] Levit, R.; de Giori, G. S.; de Moreno de LeBlanc, A.; LeBlanc, J. G. Evaluation of the effect of soymilk fermented by a riboflavin-producing Lactobacillus plantarum strain in a murine model of colitis. *Beneficial Microbes,* **2017**, *8*(1), 65-72.

[48] Levit, R.; Savoy de Giori, G.; de Moreno de LeBlanc, A.; LeBlanc, J.G. Effect of riboflavin-producing bacteria against chemically induced colitis in mice. *J. Appl. Microbiol.,* **2018**, *124*(1), 232-240.

[49] Levit, R.; Savoy de Giori, G.; de Moreno de LeBlanc, A.; LeBlanc, J.G. Protective effect of riboflavin-overproducing strain Lactobacillus plantarum CRL 2130 on 5-fluorouracil-induced intestinal mucositis in mice. *Nutrition,* **2018** (In press).