

# Effects of probiotics on immunity and iron homeostasis: A minireview

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## Narrative Review

## Effects of probiotics on immunity and iron homeostasis: A mini-review

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## SUMMARY

Iron deficiency remains a major problem in both developed and developing countries. Iron supplementation has been used as a standard intervention for the prevention and treatment of iron deficiency anemia (IDA). There are many factors affecting the efficacy, including stunting, infections or inflammations, and genetics. Recently, some studies have been conducted to further investigate the effects of probiotics on immunity and iron homeostasis. This mini review discusses about some important factors that can improve the management of IDA.

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## 1. Introduction

Anemia and stunting pose significant challenges to the health-care system as well as children's survival [1]. Despite a general decrease in global prevalence, stunting continues to affect one-third of children under the age of five in developing countries [2]. According to the 2018 Indonesian Basic Health Survey, the prevalence of stunting in Indonesia reached 31% [3].

Stunting is a chronic nutritional problem that causes growth retardation in children. It is mostly caused by poor parenting and inadequate food intake. Low intake of macronutrients and micronutrients contributes to an increase in childhood stunting. Anemia and stunting both have an impact on immune response, which impacts the duration and severity of infections or inflammations [1].

Anemia and stunting are multiple overlapping influences originating from various levels [4,5]. A study from Ethiopia showed that there was a concerning high level of anemia and stunting clustering among infants and young children [6]. In another study, 1008 children determined that severely stunted children aged 6–59

months are significantly associated with anemia [3]. In our preliminary study, we found 40 stunting patients out of a total of 240 children examined (16.6%). 26 out of the 40 stunting patients (65%) had iron deficiency and 4 (10%) had anemia of chronic diseases (ACD) [7].

Optimizing iron status can be challenging. Iron supplementation is currently a standard intervention for iron deficiency anemia (IDA). However, there are some barriers to body iron repletion via oral supplementation including underlying systemic inflammation from chronic conditions such as chronic infection or other inflammatory conditions, autoimmune diseases, renal failure, dietary factors such as phytate, and patient-reported symptoms including gastrointestinal distress. Furthermore, iron bioavailability should be considered to increase the effectiveness of iron supplementation [8,9].

The high cases of IDA require special strategies. Iron supplementation combined with vitamin C administration shows unsatisfactory result in the treatment of IDA. There are numerous studies showing the effectiveness of probiotics in enhancing iron absorption that succeed to improve of IDA [10–13].

## 2. Probiotic, immunity, and iron homeostasis

The International Scientific Association for Probiotics and Prebiotics defined probiotics as “live microorganisms that, when

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administered in adequate amounts, confer a health benefit on the host." [14] Probiotics have gained popularity due to their potential preventative and therapeutic effects, low cost, and accessible. Moreover, in this COVID-19 pandemic era, probiotics and synbiotics are a promising strategy to reduce the severity of respiratory infection symptoms, reduce the duration of disease, improve QOL, and induce and maintain remission in patients with Respiratory Tract Infection (RTI) [15]. Some studies shows that probiotics improve iron absorption [16–18].

Iron absorption is a complex process, influenced by some factors such as bioavailability, the presence of dietary enhancers, and/or inhibitors [19]. Probiotics directly affect epithelial cells by increasing the expression and excretion of mucin from goblet cells, as well as defensin, which inhibits the proliferation of pathogenic or commensal strains and affects the mucosal barrier's integrity. Probiotics also enhance tight junction stability, which reduces permeability of the pathogens and their products. Moreover, probiotics affect mucosal immunity by increasing the number of sIgA-producing cells in the lamina propria, which prevents colonization on epithelium by binding to bacteria and antigens [20–22].

The prebiotic supplement galactooligosaccharide, selectively used by commensal Bifidobacterium spp, has been shown to enhance dietary iron absorption [23]. The study also reported the increase of Bifidobacterium spp and short-chain fatty acid (SCFAs) production linked to decreased fecal pH that likely to play a role in iron enhancement. Meanwhile, fermented foods (e.g., yogurt, vegetables) have also been shown to enhance dietary iron absorption [17,24].

Immune responses that emerge after infection or inflammation enhance pro-inflammatory cytokines particularly IL-6 and TNF- $\alpha$  release and suppress the production of anti-inflammatory cytokines. IL-6 induces the hepatocytes to release hepcidin which increases the iron deposit in cells and disturbs the iron absorption in the intestines. The accumulated iron deposit in the cell leads to prolonged infection or inflammation. In addition, it will also affect T cell proliferation and maturation through iron-dependent Trf-1 [25]. This will impact on more prolonged infection or inflammation. The iron homeostasis and immune response seem to have a two-

way relationship [25]. Overcoming the infection or the inflammation is the key to maintain iron homeostasis. However, it is not easy to diagnose and treat the infection or inflammation. The longer the infection or the inflammation lasts, the longer iron homeostasis disruption will be, and vice versa. In this complex situation, probiotics might give solutions by: 1. Increasing mucin production and antimicrobial peptides (AMPS), 2. Stimulating the epithelial cell to release TGF- $\beta$  and IL-10 which induce the Treg maturation and proliferation, 3. suppressing pro-inflammatory cytokines such as IL-6 reduces hepcidin production [26,27] (see Table 1).

Lactic acid-forming bacteria, including lactobacilli, are thought to increase dietary iron bioavailability through several mechanisms such as reducing intestinal pH shifts in gut microbiota metabolism, metabolite formation, and promotion of anti-inflammatory immunomodulation [29]. This suggests that probiotic has an important role in optimizing dietary iron bioavailability and improving iron status without the gastrointestinal burden of additional supplemental iron [32].

Several studies show positive effect of *Lactobacillus plantarum* 299v on non-heme iron absorption [16]. A meta-analysis of eight studies observed that *L. plantarum* 299v significantly improved non-heme dietary iron absorption in humans [33]. The Lp299v has been shown to increase the amount of ferric iron in vitro digested meals and drinks. This, in combination with the ability of Lp299v to increase levels of a ferric reductase (duodenal cytochrome B, DcytB) in human intestinal cells (Caco-2/HT29 MTX cells), may explain the positive effect on iron absorption [18].

Lp299v improve iron status in a non-anemic, iron-deficient population when administered in combination with a low dose of iron [34]. Lp299v can suppress inflammatory parameters [35–37]. Thus, it could lead to a change in hepcidin expression and iron absorption [18]. It also might reduce GI side effects during intense exercise and/or following consumption of iron supplements. Intake of Lp299v has previously been shown to increase the expression of the activation marker CD25 on CD8+ T-cells [38].

Another cause of unsuccessful iron therapy in IDA is the presence of gene mutation [39]. Several studies have identified several gene variants related to iron balance in the body, which can cause

**Table 1**  
Mechanism of probiotics on the iron bioavailability.

Author	Year	Sampling	Mechanism
Bergqvist et al. [28]	2006	An in vitro digestion/Caco-2 cell model was applied to explore the impact of lactic acid (LA) fermentation by <i>Lactobacillus pentosus</i> and <i>Leuconostoc mesenteroides</i> .	The difference in pH before and after digestion as the acid environment favors the Fe <sup>2+</sup> rather than the Fe <sup>3+</sup> ion.
Scheers et al. [18]	2016	Human iron absorption from lactic-fermented vegetables: 17 volunteers consumed lactic-fermented vegetable with <i>Lactobacillus plantarum</i> for 2 weeks, and followed by consumed Fe solution orally for 2 weeks. Lactate and fermented vegetable experiments in the Caco-2/HepG2 cell system. <i>L. plantarum</i> , <i>L. mesenteroides</i> , and <i>L. pentosus</i> in Human Caco-2 cells (HTB37) and human HepG2 cells.	Ferric iron is more stable in an acidic water solution than ferrous iron - mucus layer binds Fe <sup>3+</sup> under acidic conditions and less reactive.
Mack et al. [29]	2003	HT29 cells grown to enhance expression of MUC3 mucins were incubated with selected <i>Lactobacillus</i> strains.	Selected probiotic <i>Lactobacillus</i> species have the ability to adhere to intestinal epithelial cells and rapidly induce eukaryotic MUC3 mucin expression. The upregulated MUC3 mucin gene product is a secreted mucin that has the ability to inhibit enteric pathogen epithelial cell adherence.
Natanzi et al. [30]	2017	In Vitro assay, using <i>Lactobacillus acidophilus</i> in Caco-2 cells	The phytases are a group of enzymes that can degrade phytic acid. Phytic acid decreased ferritin formation in the cell line.
González et al. [31]	2017	<i>L. fermentum</i> CECT5716 was grown in anaerobic conditions in a synthetic medium	<i>L. fermentum</i> excreting molecules called p-hydroxyphenyllactic acid (HPLA). HPLA effectively reduces Fe (III) to Fe (II) and in the gastrointestinal tract can mimic the functionality of the DcytB ferric-reducing protein by promoting iron uptake by enterocytes.

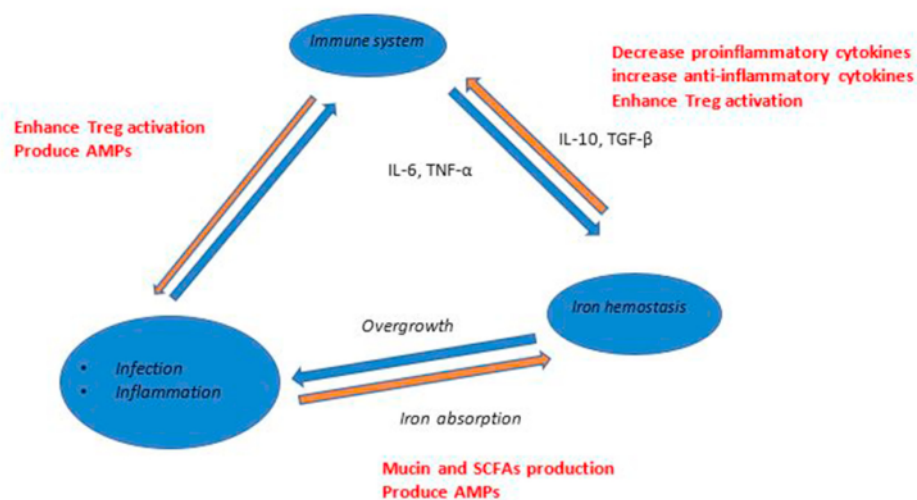


Fig. 1. Effect of probiotic on iron homeostasis (red color).

iron deficiency or overload [40,41]. The single-nucleotide polymorphism *rs29* strongly associated with lower serum iron concentration was the transmembrane protease serine 6 (TMPRSS6) gene, which produces matriptase-2, that concurrently increased the hepcidin level in the liver [42,43]. A recent study in Lombok, Indonesia showed that variant alleles (A) and (G) at *rs855791* and *rs4820268* of the TMPRSS6 gene, respectively, are associated with a lower SF concentration in iron deficiency anemia in under-two-year-old children [44].

### 3. Conclusion

The understanding of probiotics, immunity, and iron homeostasis may be the key to providing new, better iron supplementation. The effects of probiotics on iron homeostasis are summarized in Fig. 1. Future clinical trials related to a certain strain of probiotics and iron status both in healthy children and in IDA are needed.

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### Author contribution

Bahrul Fikri: Conceptualization and writing the manuscript (MS), Nadhirah Rasyid Ridha: Writing the MS, Sri Hardiyanti Putri: writing the MS, Setia Budi Salekede: Review the MS, Aidah Juliaty: Review the MS, Conny Tanjung: Conceptualization and Review the MS, Nasrum Massi: Review the MS.

### 16 Declaration of competing interest

The authors have no conflicts of interest.

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