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Supplementation of Pumpkin Seed Oil and SCFASs as a Prospective Biotherapeutic to Preserve Gut Natural Microbiota of Colitis Mice

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ABSTRACT

***Background**: Ulcerative Colitis (UC) is an inflammatory disease in the colon, due to the disruption of the interaction between the immune response and the intestinal microbiota in a genetically susceptible host. The imbalance between commensal and pathogenic intestinal microbiota promotes inflammation. This study aims to explore the efficacy of pumpkin seed oil and SCFAs in maintaining the intestinal microbiota in UC model mice. **Methods**: Four groups, K1, K2, K3, and K4, as health control given distilled water (K1); K2, K3, and K4 induced UC with 2% DSS, followed by administration of Short Chain Fatty Acids (SCFAs) (K3), supplementation of SCFAs and pumpkin seed oil (K4). The number of colonies measured microbiota diversity (log CFU/mL/g) in NA, EMB, MRSA, and McConkey media. Hemoglobin and hematocrit were also measured to assess anemia. **Results**: The average number of bacterial colonies that grew on NA, EMB, MRSA, and McConkey media in K4 was 7.65, 5.78, 7.85, and 6.20, respectively, and the average HB and hematocrit levels were 14.2 g/dL and 42%, respectively. There was greater microbiota diversity and lactobacillus bacteria in UC rats given pumpkin seed oil and SCFAs, with fewer Enterobacteriaceae and E. coli than in the UC model. The increase in HB and hematocrit levels also showed the same trend. **Conclusion**: Pumpkin seed oil at a dose of 100 mg/kg and SCFAs can be a prospective biotherapy for maintaining the balance of the natural microbiota and increasing HB levels.*

INTRODUCTION

The intestines play an important role in health. The intestines are inhabited by various groups of bacteria, archaebacteria, fungi, and viruses along the gastrointestinal tract [1]. The density of bacteria in the intestines, especially in the colon, is estimated at 1011 to 1012 per milliliter, making the colon one of the densest microbial habitats on Earth [2].

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Barrier function, metabolism, immune response, and homeostasis in the host intestine are influenced by cell signaling involving the microbiome, proliferation, and neurotransmitter synthesis [3]. The gut microbiota is crucial for inducing immune tolerance and preventing pathogen colonization through mechanisms such as nutrient competition and bactericidal production [4, 2].

Inflammatory Bowel Disease (IBD) is characterized by chronic inflammation of the intestine. It is divided into Ulcerative Colitis, which involves inflammation of the mucosa in the colon, and Crohn's Disease, which can occur in the terminal ileum [5]. The exact etiology of Ulcerative colitis is not yet fully understood. Still, it can result from disruption of the interaction between the immune response and the gut microbiota in genetically susceptible individuals [6]. The gut microbiota positively affects immune response; an imbalance between commensal and pathogenic microbiota (dysbiosis) leads to an inflammatory event.

The pathogenicity of DSS-induced US is closely related to inflammation. The inflammatory process can be reduced by Short-Chain Fatty Acids (SCFAs). SCFAs, including acetate, butyrate, and propionate sulfate, are produced mainly by the fermentation of dietary fiber by microbes in the intestine. These compounds modulate intestinal permeability [7], increase the abundance of commensal bacteria, and can be catabolized to enhance anti-inflammatory activity [6]. Pumpkin seeds are known to have various bioactive components, such as phenolic compounds and tocopherols, and are rich in protein. The linoleic acid and carotenoid content of pumpkin seed oil can act as anti-inflammatory agents, soluble fiber can act as a laxative, and tocopherol can have free radical-fighting activity [8].

Several studies have examined the use of pumpkin seed oil as a powerful antioxidant. Still, the combination of pumpkin seed oil, especially when used as a prebiotic with SCFAs, has not been studied. Pumpkin seed and SCFAs supplementation help reduce inflammation associated with colitis and increase the number of commensal bacteria in the gut, which may alleviate the disease. This study aimed to examine the effect of pumpkin seed oil and SCFAs supplementation on preserving the gut microbiota in a mouse model of ulcerative colitis. In the future, pumpkin seed oil may have potential as a natural colitis reliever, helpful in the community.

METHODS

Experimental Design

This study was an Experimental laboratory study with mice (*Mus musculus*) as the animal model of colitis. A total of 32 mice (*Mus musculus*) were randomly divided into four groups, K1, K2, K3, and K4. The animals used in this study meet the inclusion criteria, i.e., male mice, body weight range of 30-35 grams, healthy condition, and no physical defects; strain BALB-C.

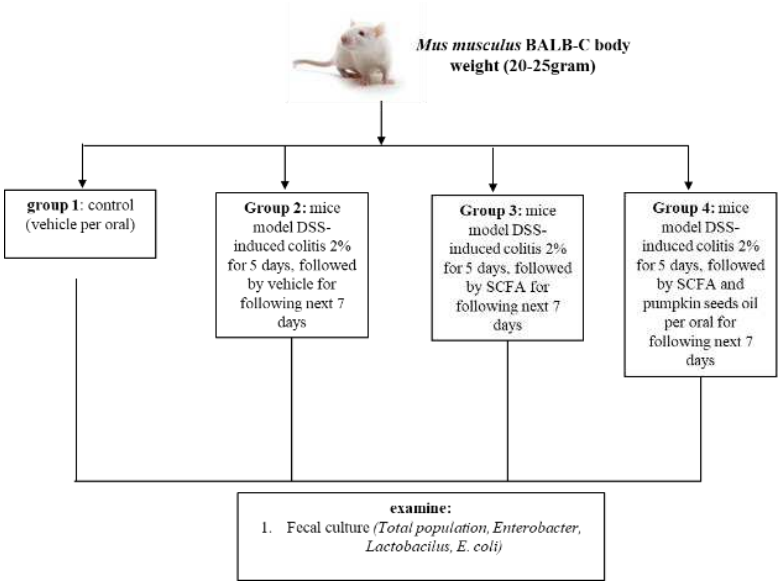

Figure 1. Experimental Design of this study

Materials and Measurement

Short Chain Fatty Acids (SCFAs) (sigma-aldrich), including sodium acetate (#S2889), propionate (#P1880), and butyrate (#B5887) (67.5 mM, 25.9 mM, and 0.946 mM) dissolved in sterile drinking water and given for 7 days (ad libitum). Dextran sulfate sodium (MP-Biomedical #216011025) 2% dissolved in sterile drinking water and given for 5 days (ad libitum). Pumpkin Seed Oil (Beworths) in the form of soft capsules with a dose of 100 mg/Kg BW per day orally. Growth media: Nutrient agar (Merck), Eosin Methylene Blue (Oxoid), MRS (de Man, Rogosa and Sharpe) agar (Merck), McConkey Agar (Merck). Hb levels (g/dL) and Hematocrit (%) were measured using test strips (Fora6) via whole blood isolated from the tails of mice shortly before termination.

Procedure

Fecal samples were collected on the 20th day after fasting for 12 hours. The bacterial culture procedure involved weighing 1 gram of fecal sample and dissolving it in 9 mL of 0.5% NaCl solution. Serial dilutions 10^{-1}-10^{-8} were prepared for each sample. Samples inoculated in selective media, including nutrient agar, are widely used for the growth of various types of bacteria, i.e, total population, Eosin Methylene Blue for Escherichia coli, de Man, Rogosa, and Sharpe for Lactobacillus, and McConkey for Enterobacteriaceae, then incubated for 24-48 hours (37 °C) [9].

Analysis

Observations consist of counting the number of colonies and the number of bacteria. The number of colonies that grew was counted. The number of colonies was calculated in colony-

forming units (CFU)/ml/gram. The calculation of CFU/ml/gram is (log dilution + log (number of colonies x feces weight) (Holota et al., 2019).

RESULTS

Administration of SCFAs and Pumpkin Seed Oil to Microbial Diversity

Bacterial diversity was observed in the growth media of Natrium Agar (NA), Eosin-Methylene Blue (EMB) agar, *Lactobacillus* MRS agar, and McConkey agar. The colony counts for each selective medium, in CFU/ml/gram, are shown in Table 1. The effect of SCFAs and Pumpkin Seed Oil on Hemoglobin and Hematocrit Levels is displayed in Table 2.

Table 1. Number of Colonies of Bacteria in Various Growth Media

Colony number (CFU/ml/ gram)				
Group	NA	EMB	MRSA	Mc Conkey
UC	7.46	6.13	7.27	5.84
K1	6.68[a]	4.21[a]	6.55[a]	5.19[a]
K2	6.97[a]	4.85[ab]	7.06[ac]	5.34[a]
K3	8.07[b]	5.50[b]	7.25[ac]	6.44[bc]
K4	7.65[ab]	5.78[b]	7.85[bc]	6.20[ac]
p value	0,005*)	0.012*)	0.030)	0.049*)

Statistical analysis with ANOVA,
*) and different superscripts showed statistical differences

On nutrient agar, SCFA supplementation yielded the highest number of colonies in the total population. The number of bacteria grown in EMB, MRSA, and McConkey represented in K1 is the lowest among the three.

Table 2. Hemoglobin and Hematocrit Levels between Groups

Group	HB Level (g/dL)	Hematocrit (%)
K1	15.4	45%
K2	13.9	41%
K3	13.8	41.8%
K4	14.2	42%
P value	0.79	0.82

Table 2 shows the average HB and Hematocrit levels in mice across various groups, with the highest values in the K1 (control) group.

DISCUSSION

Groups K1 and K2 showed no difference in the number of colonies across all bacterial populations on selective media. Still, they did differ, especially in K2, compared to K3 and K4, whose colony numbers increased gradually on NA. The number of colonies grown on EMB and

McConkey media increased from K1 to K4. The number of colonies on MRSA growth media showed no differences among groups, except for K1 and K4. Group K4 showed the highest number of colonies on MRSA media.

Eosin-Methylene Blue is a selective medium for *E. coli* and gram-negative bacteria. Meanwhile, McConkey medium is used to isolate enteric, Gram-negative bacteria (Enterobacter) and to differentiate lactose-fermenting and non-lactose-fermenting Gram-negative bacteria. The growth of *E. coli* in EMB between the colitis groups treated with SCFAs and a combination of SCFAs + pumpkin seed oil (K3, K4) did not show significant differences in colony counts. Still, it was higher than in healthy mice (K1).

Throughout the gastrointestinal system, gut bacteria are important regulators of the digestive system, while commensal bacteria are essential for the synthesis, absorption, and extraction of several nutrients and metabolites [2].

In a healthy digestive tract, a balance is required among epithelial cell integrity, the immune system, and the resident microbiota [10]. Colitis is a condition characterized by inflammation that alters microbiota diversity. In inflammatory bowel disease (IBD) and ulcerative colitis, the composition of gut bacteria is significantly altered. Proteobacteria are abundant and generally reduce the diversity of the gut microbiome. Specifically, the abundance of facultative anaerobes such as *Enterobacteriaceae, Enterococcus, and Streptococcus*. This dysbiosis is characterized by a shift towards more oxygen-consuming bacteria, a common signature in IBD [11].

Enterobacteria increase during dysbiosis due to an imbalance in the gut microbiota, often characterized by a reduction in beneficial bacteria such as *Firmicutes* and *Bacteroidetes*. This imbalance can lead to a less competitive environment, allowing opportunistic, potentially harmful bacteria such as *Enterobacteriaceae* to proliferate. Dysbiosis can be caused by various factors, including overuse of antibiotics, diet, and inflammation, which disrupt the normal microbial community and promote the growth of pathogenic bacteria. This overgrowth of *Enterobacteriaceae* can contribute to further intestinal inflammation and exacerbate conditions like inflammatory bowel disease (IBD) [10].

Administration of both SCFAs and pumpkin seed oil resulted in fewer E. coli than in the ulcerative colitis (UC) group. In this study, the SCFAs used are a combination of sodium acetate, sodium butyrate, and sodium propionate. Supplementation of SCFAs altered the composition of gut microbes, further elevating protective and commensal bacteria [12]. The efficacy of SCFAs on gut health is achieved through nurturing the integrity of the gut barrier, promoting mucus production, and reducing inflammation [13]. Maintenance of barrier integrity is pivotal for maintaining the homeostasis of the protective and pathogenic microbiota.

It was observed that administration of SCFAs (K3) and a combination of SCFAs and pumpkin seed oil significantly increased the colony number of E. coli compared to normal rats

(K1). It showed the inconsistent results. The effect of short-chain fatty acids (SCFAs) on *E. coli* growth depends on the type of SCFAs. According to Pace et al, acetate and propionate mildly inhibit the growth of adherent-invasive *E. coli* (AIEC), particularly in the stationary phase. However, butyrate does not have a significant inhibitory effect on AIEC growth. Additionally, propionate and butyrate increase the virulence characteristics of AIEC by upregulating flagellar synthesis genes and downregulating capsule assembly and transport genes, thereby enhancing AIEC's adherence to and invasion of intestinal cells [14].

Concentration of SCFAs used are sodium acetate (67.5 mM), propionate (25.9 mM), and butyrate (0.946 mM). In a healthy gut, the range of SCFAs is between 60 and 150 mM [15]. It might be that a low concentration of SCFAs is not yet sufficient to attenuate inflammation and suppress the growth of E. coli as a pathogenic bacterium. An increasing number of E. coli colonies in colitis conditions treated with SCFAs at this concentration is unable to restore the inflammatory condition, resulting in the number of *E. coli* bacteria remaining high.

Supplementation of pumpkin seed oil, added together with SCFAs, in colon inflammation conditions showed no difference in the number of enterobacter colonies compared with the healthy mice group (K1). SCFAs themselves have an anti-inflammatory effect.

The gut barrier comprises a mucus layer, commensal microbiota, and a single layer of epithelium. Pumpkin seeds are one of the natural foods included in low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FOMAP) [16]. *Cucurbita* sp. consists of many nutritious ingredients, particularly in the seeds, such as carotenoids, poly-mono unsaturated fatty acids (PUFA-MUFA), tocoferol, soluble fiber, etc. [8].

MRS agar is a selective medium for the growth of lactic acid bacteria. *Lactobacillus* and the digestive tract microbiota are a group of lactic acid bacteria. *Lactobacillus* in the digestive tract may both protect against pathogens and provide the host with the substrate it needs; the relationship between Lactobacillus and the host is mutualistic. By secreting mucus, this bacterium is also known to strengthen the intestinal barrier [17]. *Lactobacillus* is a genus of beneficial bacteria commonly found in the gut microbiota. It is known for its role in maintaining gut health by producing lactic acid, which helps inhibit the growth of harmful bacteria. In IBD patients, the overall diversity of gut microbiota is often reduced, and there is typically a decrease in beneficial bacteria, including *Lactobacillus*. This reduction can contribute to the dysbiosis and inflammation characteristic of IBD [18].

The effect of SCFAs and pumpkin seed oil on the increase in the number of Lactobacillus seems clear in K4. The number of Lactobacillus colonies was significantly higher in K4 than in other groups, specifically after the colitis state or inflammation. A combination of SCFAs and pumpkin seed oil has a double positive effect on inflammation, as it has highly antioxidant properties.

The number of bacterial colonies growing on NA, EMB, MRSA, and Mc Concey media differed significantly between groups (p value <0.005).

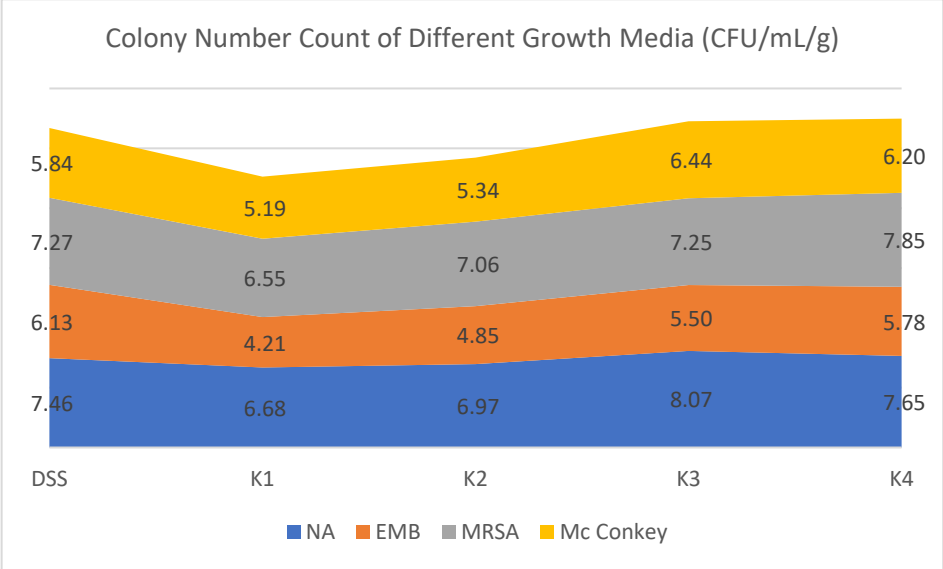

Figure 1. Colony number count of bacteria in different growth media

Hemoglobin and hematocrit levels did not differ among the four groups, but colitis mice showed a slight decrease in Hb and Hct compared with the control group. Again, SCFAs and pumpkin seed oil can reverse the inflammatory condition, resulting in a gradual increase in Hb and Hct levels.

K3 and K4 represent colitis model mice that were treated with SCFAs and the combination. The highest hemoglobin levels among in K4 and the lowest in K3, similar to the % hematocrit, but there was no significant difference between the groups in hemoglobin levels and hematocrit percentage, this is because the administration of SCFAs (K3) and a combination of SCFAs + pumpkin seed oil (K4) supplements for 7 days was able to increase Hb levels and % hematocrit to normal levels even though they had experienced inflammation due to DSS administration. Likewise, the administration of distilled water for 7 days after DSS administration normalized Hb levels and % hematocrit, which did not differ in K1.

Colitis is characterized by mucosal inflammation, which leads to gastrointestinal disruption, dietary restriction, including iron malabsorption, and, in turn, anemia [19]. Hemoglobin level and Hematocrit below normal are categorized as anemia or may be a trait of chronic inflammatory disorders associated with IBD patients [20]. Further research is needed regarding the role of SCFA and pumpkin seed oil, especially in varying degrees of colitis severity in mice induced by DSS at various doses and treatment durations, in the abundance of the intestinal microbiota.

It is critical to recognize the limitations of this study. Firstly, dose 2% DSS may vary in its degree of colitis across mice, so the degree of colitis may not be uniform. Secondly, it is necessary

to include a group with only colitis as a negative control, which, unfortunately, was not included in the design of this study. Administration of different doses of DSS may provide a more reliable profile of colitis activity. This finding contributed to understanding the potential of SCFA combined with pumpkin seed oil in colitis, which, to the best of our knowledge, has never been studied before.

CONCLUSION

The objective of this study is to explore the efficacy of pumpkin seed oil and SCFAs on the maintenance of intestinal microbiota in the UC model mice. A combination of SCFAs and pumpkin seed oil can increase the number of Lactobacillus in the gut microbiota. It can reduce the number of pathogenic bacteria such as Enterobacter and E. coli. Further, the combination helps improve red blood cell parameters, including hemoglobin and hematocrit levels.

DECLARATIONS

Ethics approval

This study has received ethical approval with number 96/SLE/FK/UWKS/2024 through the ethics committee of the Faculty of Medicine, Wijaya Kusuma University, Surabaya.

Conflict of interest

The authors declare no conflict of interest.

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REFERENCES

[1] Matei-Lațiu MC, Gal AF, Rus V, Buza V, Martonos C, Lațiu C, et al. Intestinal Dysbiosis in Rats: Interaction between Amoxicillin and Probiotics, a Histological and Immunohistochemical Evaluation. Nutrients 2023;15:1–14. https://doi.org/10.3390/nu15051105.

[2] Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms 2019;7. https://doi.org/10.3390/microorganisms7010014.

[3] Wen X, Xie R, Wang HG, Zhang MN, He L, Zhang MH, et al. Fecal microbiota transplantation alleviates experimental colitis through the Toll-like receptor four signaling pathway. World Journal of Gastroenterology 2023;29:4657–70. https://doi.org/10.3748/wjg.v29.i30.4657.

[4] Lin MK, Yang YT, Lin LJ, Yu WH, Chen HY. Pulsatilla decoction suppresses matrix metalloproteinase-7-mediated leukocyte recruitment in dextran sulfate sodium-induced colitis mouse model. BMC Complementary Medicine and Therapies 2022;22:1–14. https://doi.org/10.1186/s12906-022-03696-w.
[5] Meers GK, Bohnenberger H, Reichardt HM, Lühder F, Reichardt SD. Impaired resolution of dss-induced colitis in mice lacking the glucocorticoid receptor in myeloid cells. PLoS ONE 2018;13:1–15. https://doi.org/10.1371/journal.pone.0190846.
[6] Li L, Peng P, Ding N, Jia W, Huang C, Tang Y. Oxidative Stress, Inflammation, Gut Dysbiosis: What Can Polyphenols Do in Inflammatory Bowel Disease? Antioxidants 2023;12:1–27. https://doi.org/10.3390/antiox12040967.
[7] Tan C, Wu Q, Wang H, Gao X, Xu R, Cui Z, et al. Dysbiosis of Gut Microbiota and Short-Chain Fatty Acids in Acute Ischemic Stroke and the Subsequent Risk for Poor Functional Outcomes. Journal of Parenteral and Enteral Nutrition 2021;45:518–29. https://doi.org/10.1002/jpen.1861.
[8] Nascimento R de P do, Moya AMTM, Machado AP da F, Geraldi MV, Diez-Echave P, Vezza T, et al. Review on the potential application of non-phenolic compounds from native Latin American food byproducts in inflammatory bowel diseases. Food Research International 2021;139. https://doi.org/10.1016/j.foodres.2020.109796.
[9] Holota Y, Dovbynchuk T, Kaji I, Vareniuk I, Dzyubenko N, Chervinska T, et al. The long-term consequences of antibiotic therapy: Role of colonic short-chain fatty acids (SCFA) system and intestinal barrier integrity. PLoS ONE 2019;14:1–25. https://doi.org/10.1371/journal.pone.0220642.
[10] Monteros MJM, Galdeano CM, Balcells MF, Weill R, De Paula JA, Perdigón G, et al. Probiotic lactobacilli as a promising strategy to ameliorate disorders associated with intestinal inflammation induced by a non-steroidal anti-inflammatory drug. Scientific Reports 2021;11:1–15. https://doi.org/10.1038/s41598-020-80482-z.
[11] Chang CS, Liao YC, Huang CT, Lin CM, Cheung CHY, Ruan JW, et al. Identification of a gut microbiota member that ameliorates DSS-induced colitis in intestinal barrier enhanced Dusp6-deficient mice. Cell Reports 2021;37. https://doi.org/10.1016/j.celrep.2021.110016.
[12] Lee JG, Lee J, Lee A reum, Jo SV, Park CH, Han DS, et al. Impact of short-chain fatty acid supplementation on gut inflammation and microbiota composition in a murine colitis model. Journal of Nutritional Biochemistry 2022;101:108926. https://doi.org/10.1016/j.jnutbio.2021.108926.
[13] Silva YP, Bernardi A, Frozza RL. The Role of Short-Chain Fatty Acids from Gut Microbiota in Gut-Brain Communication. Frontiers in Endocrinology 2020;11:1–14. https://doi.org/10.3389/fendo.2020.00025.
[14] Pace F, Rudolph SE, Chen Y, Bao B, Kaplan DL, Watnick PI. The Short-Chain Fatty Acids Propionate and Butyrate Augment Adherent-Invasive Escherichia coli Virulence but Repress Inflammation in a Human Intestinal Enteroid Model of Infection. Microbiology Spectrum 2021;9:1–12. https://doi.org/10.1128/spectrum.01369-21.
[15] Zhang S, Dogan B, Guo C, Herlekar D, Stewart K, Scherl EJ, et al. Short chain fatty acids modulate the growth and virulence of pathosymbiont escherichia coli and host response. Antibiotics 2020;9:1–20. https://doi.org/10.3390/antibiotics9080462.
[16] Liang L, Saunders C, Sanossian N. Food, gut barrier dysfunction, and related diseases : A new target for future individualized disease prevention and management 2023:1671–704. https://doi.org/10.1002/fsn3.3229.
[17] Dempsey E, Corr SC. Lactobacillus spp. for Gastrointestinal Health: Current and Future Perspectives. Frontiers in Immunology 2022;13:1–15. https://doi.org/10.3389/fimmu.2022.840245.
[18] Baldelli V, Scaldaferri F, Putignani L, Del Chierico F. The role of enterobacteriaceae in gut microbiota dysbiosis in inflammatory bowel diseases. Microorganisms 2021;9:1–15. https://doi.org/10.3390/microorganisms9040697.

[19] Hsiao PY, Weng MT, Chang CH, Huang LY, Tung CC, Leong YL, et al. Anemia in inflammatory bowel disease course is associated with patients' worse outcome. Journal of the Formosan Medical Association 2023;122:549–56. https://doi.org/10.1016/j.jfma.2022.11.004.
[20] Rieder F, Paul G, Schnoy E, Schleder S, Wolf A, Kamm F, et al. Hemoglobin and hematocrit levels in the prediction of complicated Crohn's disease behavior - A cohort study. PLoS ONE 2014;9. https://doi.org/10.1371/journal.pone.0104706.