BIODIVERSITAS
Volume 23, Number 5, May 2022
Pages: 2304-2310

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230508

The potential of *Lactobacillus buchneri* isolated from spontaneous rabbit meat fermented-bekasam against *Salmonella typhimurium* by in vivo evaluation

EKA WULANDARI[1,♥], HUSMY YURMIATI[2], TOTO SUBROTO[3], OKTA WISMANDANU[4], SHAFIA KHAIRANI[4]

[1]Departement of Livestock Product Technology, Faculty of Animal Husbandry, Universitas Padjadjaran. Jl. Raya Bandung Sumedang Km. 21, Sumedang 45363, West Java, Indonesia. Tel./fax.: +62-22-7798247, ♥email: eka.wulandari@unpad.ac.id
[2]Departement of Livestock Production, Faculty of Animal Husbandry, Universitas Padjadjaran. Jl. Raya Bandung Sumedang Km. 21, Sumedang 45363, West Java, Indonesia
[3]Departemen of Chemistry, Faculty of Mathematics and Science Universitas Padjadjaran. Jl. Raya Bandung Sumedang Km. 21, Sumedang 45363, West Java, Indonesia
[4]Program of Veterinary Medicine, Faculty of Medicine, Universitas Padjadjaran. Jl. Raya Bandung Sumedang Km. 21, Sumedang 45363, West Java, Indonesia

Manuscript received: 5 March 2022. Revision accepted: 19 April 2022.

Abstract. *Wulandari E, Yurmiati H, Subroto T, Wismandanu O, Khairani S. 2022. The potential of* Lactobacillus buchneri *isolated from spontaneous rabbit meat fermented-bekasam against* Salmonella typhimurium *by* in vivo *evaluation. Biodiversitas 23: 2304-2310.* Rabbit meat bekasam is a traditional fermented food and is considered a healthy and functional food due to its nutrient content and microorganism. Lactic acid bacteria (LAB) was dominant microorganism and *Lactobacillus buchneri* E3 is one of LAB isolated during fermentation of the bekasam. *L. buchneri* E3 has antimicrobial activity against pathogen bacteria. The present study aimed to investigate in vivo assessment of antimicrobial activity against *Salmonella typhimurium* using BALB/c mice. Oral administrations of three doses of *L. buchneri* E3(10^8, 10^9, and 10^{10} CFU/day/mouse) were performed for seven consecutive days. On the 8th day, each animal was inoculated with a single *S. typhimurium*, and on the 13th day, the mice were sacrificed for observation. The result showed that oral administration of *L. buchneri* E3 significantly increased the total population of LAB, significantly decreased *S. typhimurium* populations in the intestines, liver, and spleen, while increasing beneficial bacterial population and maintaining the normal hematology in the mice. The probiotic also maintained the histological examination of spleen and liver. These suggest that *L. buchneri* E3 is safe and could be used as the starter for fermentation products.

Keywords: BALB/c, enteropathogenic, in vivo, *Lactobacillus buchneri* E3, probiotic

INTRODUCTION

Salmonella is one of the enteropathogenic bacteria that cause diseases in humans (food-borne diseases) and imposes serious issues in the community with an implication on the socio-economic sector (Grace 2015). Enteropathogenic bacteria are the prevalent infectious bacteria in some developing countries (Mare et al. 2011). *Salmonella* was found especially in animal products due to poor handling or processing and infected digestive organs. Treatment of *Salmonella* can be done with antibiotics or consuming materials that can inhibit *Salmonella* activity in the gastrointestinal tract. These materials have to survive in the digestive tract. One of the potential ingredients that can reduce *Salmonella* activity in the gastrointestinal tract is probiotics which have antibacterial activity (Steinberg et al. 2014). Probiotics can be obtained from various sources in nature.

Probiotic is living bacteria that contribute positively to health. One of the criteria for probiotics is producing antimicrobial substances that inhibit the growth of enteric bacterial pathogens (Hill et al. 2014). The species of *Lactobacillus* and *Bifidobacterium* strains are the most investigated bacterial strains and have proven to have beneficial probiotics. These bacteria can relieve gastrointestinal disease by maintaining the balance of intestinal microflora. The probiotics mechanism to maintain a balance microbiota population in the host intestine is through nutrition competition, receptor competition for epithelial cell attachment, the production of antimicrobial compounds, and immunity stimulations (Azad et al. 2018). *Lactobacillus buchneri* E3 is one of beneficial LAB isolated from fermented rabbit meat bekasam with the efficient and potential probiotic attribute. Bekasam is originally made from fish and is widely known in Indonesia especially in South Sumatra and Central Kalimantan. Rabbit meat has a superior quality of nutrition with high protein, therefor today bekasam is also made using rabbit meat. *L. buchneri* E3 has antibacterial activity against pathogen bacteria *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* and *Salmonella typhimurium* (Wulandari et al. 2020).

It is necessary to study the safety assessment on *L. buchneri* E3 as they are intended to be dietary or food supplements for human. Thus, this study aimed to

investigate the ability of as a probiotic to decrease the number of pathogenic bacteria *S. typhimurium* using experimental BALB/c mice.

MATERIALS AND METHODS

Bacterial preparation

Lactobacillus buchneri E3 was isolated from rabbit meat bekasam. The isolate was purified and identified molecularly using 16S rRNA gene sequencing and BLASTn analysis. The bacterial suspension was prepared by growing *L. buchneri* E3 in the deMan Rogosa Sharpe (MRS) broth medium and incubating the isolates at 37ºC for 24 hr. Then, the suspensions were centrifuged 8,848 g for 10 min at 4ºC. After the centrifugation, the supernatant was separated, and the pellet was cleaned three times using deionized water. The suspension of *L. buchneri* E3 bacteria was prepared using sterile saline as the solvent (Shokryazdan et al. 2016). The *Salmonella typhimurium* ATCC 14088 were derived from the Central Laboratory of Padjadjaran University, Bandung, Indonesia. The study design was approved by the ethics committee of Universitas Padjadjaran, Indonesia.

In vivo model with *Salmonella typhimurium*

The 20 male BALB/c mice aged 6-8 wk and weighed 22-32 g derived from PT Biofarma, Bandung, Indonesia, were used in present investigation. The mice were acclimatized for seven days in the experimental cage (three mice per cage) at 22 ± 2ºC, 12 hr day/night cycle). The standard ratio offered to the mice contained 13% water, 10-12% protein, 5% fat, 8% fiber, 14% ash, 3% calcium and 0.7% phosphor. The mice were divided into five groups to examine the effect of probiotics administered as follows: Group 1 (X1): 0.2 mL of NaCl physiological solution, (negative control), Group 2 (X2): 0.2 mL of *Bifidobacterium bifidum* [1×10^8CFU/mL] (positive control), Group 3 (X3): 0.2 mL of *L. buchneri* E3 [1×10^7 CFU/mL], Group 4 (X4): 0.2 mL of *L. buchneri* E3 [1×10^8 CFU/mL] and Group 5 (X5): 0.2 mL of *L. buchneri* E3 [1×10^9 CFU/mL].

The feed was offered orally according to treatments for seven days using a method by Mohamed et al. (2010) and Giles-Gómez et al. (2016). On the 8th days, the mice were infected with *S. typhimurium* (0.2 mL containing 1×10^7 CFU/mL dissolved in physiological NaCl). On the 12th days, the blood sample was drawn from the mice to measure the hematological parameter using a tube pre-filled with K_2-EDTA and analyzed using an automated hematological analyzer. After the mice were sacrificed, the intestinal organs, liver, and spleen were removed aseptically, dissolved in demineralized water up to 5 mL, and homogenized using a vortex. A serial dilution was performed on the cell suspension to *Xylose-Lysine Deoxycholate Agar* (XLDA) medium and MRS agar medium to enumeration *Salmonella* and LAB respectively and incubated at 37°C for 24 hr.

Histopathology analysis and scoring

Spleen and liver tissue were processed and histological preparations were made by staining Hematoxylin Eosin (HE). Histopathological changes were analyzed by microscope.

Spleen and liver histopathological preparation were observed under a microscope with 100X magnification. The spleen was examined histopathologically scored based on the presence of hemorrhage and necrosis. The results of hemorrhage examination were given a score of 0 (normal, no bleeding), 1 (light/focal bleeding), 2 (moderate/multifocal bleeding), 3 (heavy/diffuse bleeding). The results of the necrosis examination are 0 (no necrosis), 1 (mild/focal necrosis), 2 (moderate/multifocal necrosis), 3 (severe/diffuse necrosis).

The liver was examined histopathologically scored based on the presence of congesty, necrosis and inflammation. The score for congestion was a score of 0: no congestion; score 1: focal congestion (mild); score 2: multifocal (moderate) congestion; score 3: diffuse congestion (heavy). The score for inflammation is 0: no inflammation; score 1: focal inflammation(light); score 2: multifocal inflammation (moderate); score 3: diffuse inflammation (severe). Score for necrosis, score 0: no necrosis; score 1: focal necrosis (mild); score 2: multifocal (moderate) necrosis; score 3: diffuse necrosis (severe).

Data analysis

The bacterial and hematological data were analyzed using analysis of variance and Duncan multiple range test (DMRT) was applied to determine the differences among treatments. Differences were considered significant at $p<0.05$. Data obtained from scoring histopathology were analyzed using the Kruskal-Wallis non-parametric statistical and the Mann-Whitney test to determine the differences between treatments. Data are expressed as means ± standard error. All statistical analyses were performed using SPSS ver. 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences were considered significant if $p < 0.05$. Analysis of variance and duncan test were used to analyze bacterial and hematological data. Kruskal-Wallis and Mann-Whitney test were used to analyze scoring histopathology using SPSS version 18.

RESULTS AND DISCUSSION

Total population of LAB and *Salmonella typhimurium* in the intestines

Total lactic acid bacterial population in the intestines illustrates the total LAB attached to the intestinal mucosa. The ability of LAB to attach to the intestines is a crucial prerequisite for probiotics. *Salmonella* can cause damage to intestinal microvilli causing impaired absorption of nutrients and inhibiting growth. *Salmonella* infection causes damage to the intestinal microvilli because of the proteolytic activity (Eng et al. 2015). The attachment of pathogenic bacteria to intestine results in colonization, cell damage, disruption of cell regulatory mechanisms, growth and intracellular reproduction (Lochine et al. 2015).

Table 1. The effect of probiotic administration on the total intestinal LAB and *Salmonella typhimurium*

Probiotic treatments	Colon		Ileum		Duodenum	
	LAB (log CFU/g)	***Salmonella typhimurium* (log CFU/g)**	**LAB (log CFU/g)**	***Salmonella typhimurium* (log CFU/g)**	**LAB (log CFU/g)**	***Salmonella typhimurium* (log CFU/g)**
X1	3.51^{d}	6.64^{a}	3.75^{c}	6.81^{a}	3.63^{d}	6.56^{a}
X2	7.51^{b}	3.72^{c}	6.75^{b}	5.79^{c}	6.18^{b}	4.80^{c}
X3	5.61^{c}	5.63^{b}	6.57^{b}	6.62^{b}	5.72^{c}	5.26^{b}
X4	5.76^{c}	5.62^{b}	6.26^{b}	6.43^{b}	5.88^{c}	4.30^{b}
X5	7.63^{a}	3.72^{c}	7.89^{a}	5.72^{c}	7.62^{a}	3.71^{c}

Note: $^{a-c}$ Mean in the same row with different superscripts differ significantly ($p < 0.05$)

The highest population of *L. buchneri* E3 showed the same effect on decreasing *Salmonella* with the X2 (*Bifidobacterium*) treatments (Table 1). This condition shows that *L. buchneri* E3 was able to pass various barrier in the digestive tract, including low pH (in the stomach) and the presence of bile salts in the intestine afterward reaching colon, ileum and duodenum. This proves that *L. buchneri* E3 able to adapt and live in the digestive tract. *L. buchneri* E3 produced bacteriocin, buchnericin, that could inhibit the growth of *Salmonella*. *Bifidobacterium* (X2) can reduce *Salmonella* translocation and dissemination by decreasing adhesion and invasion and competing for nutrients within the intestinal lumen (Aljahdali et al. 2020).

Lactobacillus buchneri E3 is reported to exhibit *in vitro* bactericidal activities that inhibit the growth of *S. aureus*, *L. monocytogenes*, *E. coli*, *S. typhimurium* (Wulandari et al. 2020). Supplementing probiotics could reduce the number of *Salmonella* in the mice because the LAB cells affected *Salmonella* by competing to attach to the epithelial cells of the intestines. Similarly, Vieco-Saiz et al. (2019) report that lactic acid bacteria can prevent the adhesion of pathogenic bacteria by reducing colonization and relieving infection. According to Shah et al. (2021), probiotics can keep the balance of the colonic microflora through colonization resistance. Colonization resistance can cause inhibit colonization by other bacteria, through competition in the nutrition or attachment site, decrease in pH, and production of antimicrobial components.

The total *Salmonella typhimurium* bacterial population in the liver and spleen

The liver is a vital organ in the body that plays an important role in the body's metabolism, filtering and processing toxic materials and then removing them from the body. Liver function and health are usually due to metabolism conditions, toxic substances, microbial infection, and circulatory and neoplasm disturbance (Robinson et al. 2016). The spleen is the largest lymphatic organ in the body. Lymphocytes destroy pathogens and macrophages ingesting and digesting cells (Lewis et al. 2019). One of the functions of spleen is to produce white blood cells and contributes to the body's immune system. Infection by *Salmonella* occurs in the digestive tract and then spreads to the liver and spleen. Accordingly, this study investigated the effect of probiotic and bioactive peptides in the liver and spleen to illustrate the infection process in the mice that indicated a competition rendered by the supplemented probiotic.

Mice offered with 10^9 CFU/mL of *L. buchneri* E3 were significantly lower than all *L. buchneri* E3 treatments in liver and spleen, as well as the positive and negative control (Table 2). It shows that *L. buchneri* E3 can survive and thrive in the digestive tracts therefore *S. typhimurium* is reduced from the intestinal cells because *L. buchneri* E3 produces antimicrobial substances. According to Monika et al. (2021), probiotics produce antimicrobial compounds such as organic acid and bacteriocin. Administration of *L. buchneri* E3 reduces the infection in the liver and spleen and stimulates the immune system. *L. buchneri* E3 can be recommended for consumption and for competing (and eventually inhibiting) the growth of *S. typhimurium*. It was in line with Giles-Gomez et al. (2016) that *L. mesenteroides* P45 could reduce *S. enterica* serovar *typhimurium* in BALB/c liver and spleen.

Haematological profile of mice

Haematological parameter is associated with the blood and blood-forming organs. Hematological parameters that include hematocrit, hemoglobin, total erythrocyte and leucocyte are feasible indicators of toxicity and possess a wide range of potential application to observes the environmental condition. Furthermore, hematological examination is a method to detect the health status that might be overlooked during the physical examination but apparently affects the fitness of the animals (Akin-Osanaiye et al. 2015).

Table 2. Total *Salmonella typhimurium* population in the liver and spleen

Probiotic treatments	***Salmonella typhimurium* in liver (log CFU/g)**	***Salmonella typhimurium* in spleen (log CFU/g)**
X1	4.61^{a}	4.60^{a}
X3	4.51^{b}	3.48^{b}
X4	3.97^{c}	3.48^{b}
X2	3.90^{c}	3.90^{c}
X5	3.64^{d}	3.54^{d}

Note: $^{a-c}$ Mean in the same row with different superscripts differ significantly ($p < 0.05$)

Table 3. The hematological profile of mice receiving probiotic treatment

Treatments	Erytrcyt (10^6/mm^3)	Leucosit (10^3/µL)	Hemoglobin (g/dL)	Trombocyte (10^3/L)	Hematocyte (%)	Limphocyte (%)	Monocyte (%)	Neutrophil (%)	Eisinophil (%)
X1	7.03^a	9.05^a	13.63^a	358.87^a	36.41^a	70.32^b	2.20^a	25.75^a	3.50^a
X2	7.25^a	7.25^b	13.57^a	338.22^a	36.21^a	77.00^a	2.07^a	26.18^a	3.08^a
X3	7.28^a	8.85^a	13.25^a	345.31^a	36.12^a	70.54^b	2.15^a	26.32^a	3.53^a
X4	7.35^a	7.25^b	13.50^a	335.56^a	36.32^a	73.97^a	2.28^a	26.60^a	3.47^a
X5	7.47^a	7.00^b	13.55^a	324.45^a	35.87^a	75.22^a	2.40^a	26.75^a	3.06^a

Note: $^{a-c}$ Mean in the same row with different superscripts differ significantly ($p < 0.05$)

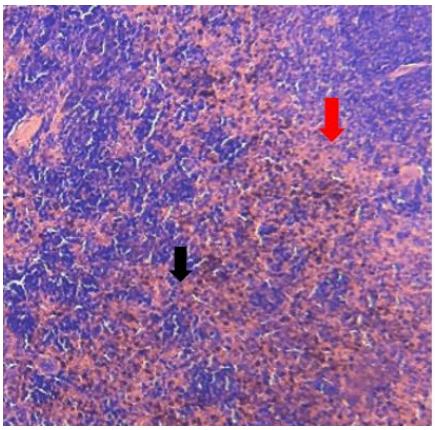

Figure 1. Histopathology of mice spleen (X1) present multifocal bleeding (red arrow), multifocal necrosis (black arrow) (HE,100x)

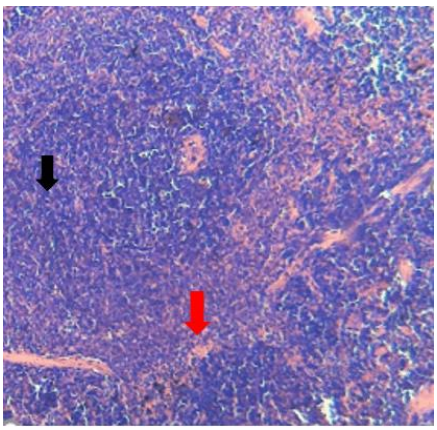

Figure 2. Histopathology of mice spleen (X2) present multifocal bleeding (red arrow), local necrosis (black arrow) (HE,100x)

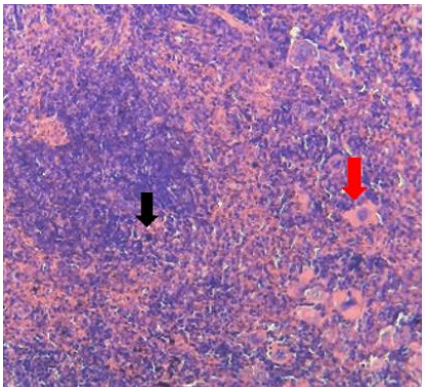

Figure 3. Histopathology of mice spleen (X3) present local bleeding (red arrow), local necrosis (black arrow) (HE,100x)

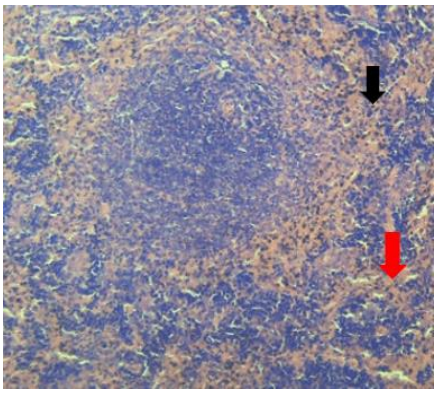

Figure 4. Histopathology of mice spleen (X4) present local bleeding (red arrow), local necrosis (black arrow) (HE,100x)

The total erythrocyte value were no significant differences among the treatments (Tabel 3). All treatments showed normal value of erythrocyte (normal range 6.93 ×10^6/mm^3-10.09 × 10^6/mm^3). Erythrocytes are the most abundant type of blood cell and carry oxygen throughout body tissues of vertebrae. The contributing factors to the total erythrocyte include age, sex, physical activity, nutrition intake, blood volume, and environmental condition.

The total leukocyte in the experimental mice offered with probiotics increases, especially in the positive control X2, X4 and X5. It is perceived that the probiotics function as the immunomodulator (immunostimulants) that could increase body immune system (Azad et al. 2018). The probiotic bacteria are attached to the intestinal surface to increase the protection of the host's digestive tracts. Additionally, probiotics can protect the host from the colonization of pathogenic bacteria with different mechanisms (La Fata et al. 2018). Increase in leukocytes is caused by leukocytes active against infection in the body. Existence infection will stimulate the release of hormones adrenals that affect the increase in circulating leukocytes. Leukocytes have two functions destroying the attacking agent with phagocytosis and producing antibodies (Nicholson 2016). The normal level of leukocytes in mice is 2×10^3-10×10^3/µL, total leukocyte that exceeds the normal threshold indicates infection. The increased number of leukocytes in mice offered with probiotics is because the probiotics serve as an immunomodulator (immunostimulant) that could improve body immune (Azad et al. 2018). Probiotics could stimulate the activation of epithelial cells and lymphocyte functions, thus improving the protection capacity of the mucosa defense system (La Fata et al. 2018).

The hemoglobin levels of mice across treatments are not significantly different and still within the normal range, 12.6-15.6g/dL. Blood transports oxygen and carries carbon dioxide from the heart to the lungs, transfers nutrition across body, and maintains body immune. Blood also contributes to the process of physiological setting and diagnosing disease or pathology in an animal (Raabe et al. 2011).

Hematocrit is the ratio between the total erythrocyte to blood plasm. The normal hematocrit value of mice is 35%-52% (Raabe et al. 2011). All treatments in this study showed non-significantly different total hematocrit across treatments. Lymphocyte titers of *L. buchneri* (X3 and X4) treatment were significantly higher than negative control (X1), this was in line with Mikulic et al. (2017) that probiotic can increase lymphocyte secretion of antibodies and produce foreign IgA. The total monocyte and eosinophile are not significantly different across treatments. Monocyte exhibits phagocytosis function and impairs foreign objects or dead tissues by using the foreign particles to improve body immune, like macrophage when responding to antigen (Hirayama et al. 2019). Eosinophils control and decrease the occurrence of hypersensitivity, respond to disease or allergy, and detox harmful substances or toxins generated by parasite or bacteria (Ramirez et al. 2018).

Histopathological examination

The result showed moderate bleeding in the group of X1 mice that were not treated with probiotics was statistically significantly different from the other treatments (there were moderate and normal bleeding) (Table 4, Figure 1-5).

Observations showed that bleeding (hemorrhage) occurred in all treatments without probiotics showing the most severe bleeding, while the percentage of spleen cell necrosis of mice with probiotic treatment was significantly lower than mice that were not treated with probiotics. This may be caused by the spleen itself is an organ that functions to filter blood and is function removing unnecessary materials from the blood such as damaged red blood cells, besides the spleen is an organ that plays a role in mobilizing blood when physiological activity increases. These findings were in line with Shokryazdan (2016) that there was no inflammatory or cellular changes were observed in the spleen of the treated rat with *L. buchneri* and *L. fermentum*. The spleen is an organ of defense against infection with foreign particles that enter through the blood (Bronte and Pittet 2013). Infection with foreign particles that enter the blood can lead to sepsis to necrosis. This bleeding can also be caused by trauma to the mice used, causing bleeding in the spleen.

Table 4. The histopathological of spleen BALB/c mice receiving probiotic treatment

Sampel	Hemorrhage	Necrosis
X1	2.60 ± 0.55^{a}	2.40 ± 0.89^{a}
X2	1.80 ± 0.45^{b}	1.00 ± 0.55^{b}
X3	1.00 ± 0.71^{c}	0.80 ± 0.45^{b}
X4	1.00 ± 0.71^{c}	0.80 ± 0.45^{b}
X5	0.60 ± 0.55^{d}	0.60 ± 0.55^{b}

$^{a-d}$ Mean in the same row with different superscripts differ significantly ($p < 0.05$)

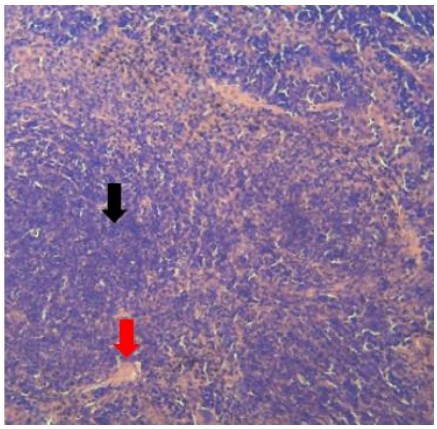

Figure 5. Histopathology of mice spleen (X5) present local bleeding and local necrosis (black arrow) (HE,100x)

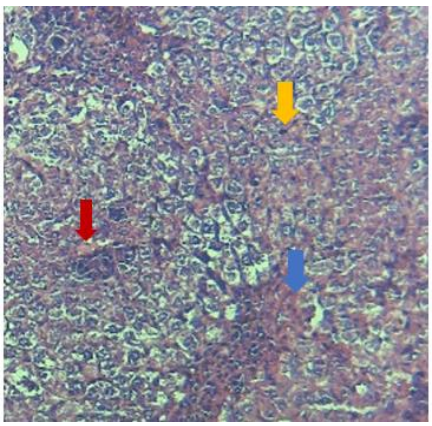

Figure 6. Histopathology of mice heart X1 present multifocal congestion (blue arrow), multifocal necrosis (red arrow) and multifocal inflammation (yellow arrow) (HE,100x)

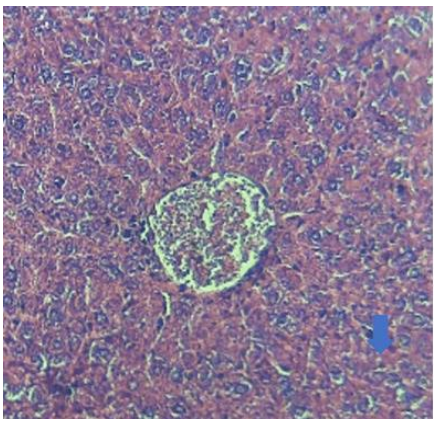

Figure 7. Histopathology of mice heart (X2) present multifocal congestion (blue arrow) (HE,100x)

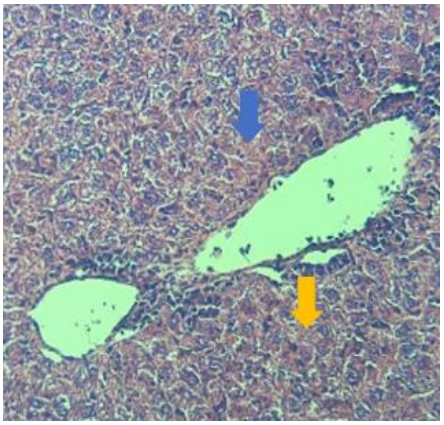

Figure 8. Histopathology of mice heart (X3) present focal congestion (blue arrow) and focal inflammation (yellow arrow) (HE,100x)

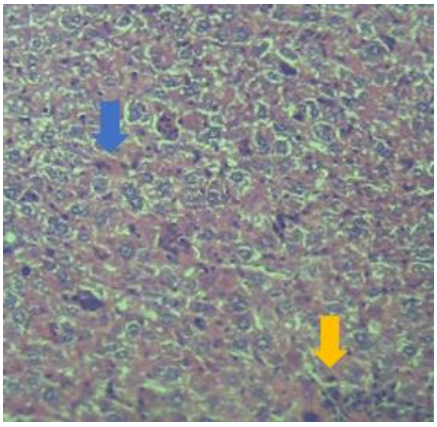

Figure 9. Histopathology of mice heart (X4) present focal congestion (blue arrow) and focal inflammation (yellow arrow) (HE,100x)

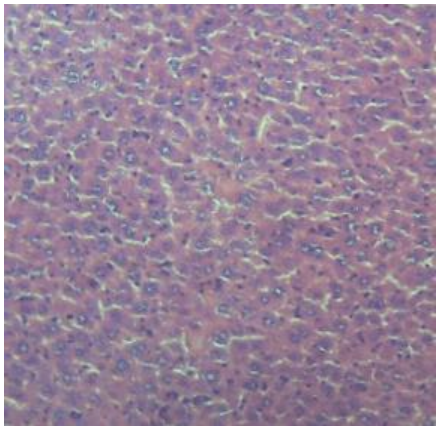

Figure 10. Histopathology of mice heart (X5) present no congestion, no necrosis and no inflammation (HE,100x)

Table 5. The Histopathological of liver BALB/c mice receiving probiotic treatment

Sampel	Congesti	Necrosis	Inflammation
X1	2.60±0.55[a]	2.40±0.89[a]	2.20±0.45[a]
X2	1.80±0.45[b]	0.40±0.55[b]	0.80±0.45[c]
X3	1.00±0.71[b]	0.80±0.45[b]	1.20±0.45[b]
X4	1.00±0.71[b]	0.80±0.45[b]	1.80±0.45[b]
X5	0.40±0.55[c]	0.40±0.55[b]	0.40±0.55[c]

Note: [a-c] Different superscripts in the same row represent significant differences ($p < 0.05$)

The results of observations with a microscope on the liver of mice showed that there were variations in the histopathological changes of mice. Based on the observations, it was found that there was moderate (multifocal) congestion in mice without probiotic treatment, whereas in the probiotic treatment there was mild congestion, while the highest concentration of probiotics was not found in the X5 treatment (Table 5, Figure 6-10). Congestion is a lesion that describes circulatory disturbances and can also be an indicator of network repair. Congestion is the accumulation of blood in the veins due to slowed or even stopped blood flow. Causes of congestion include presence of obstruction and stenosis (Hilscher and Sanchez 2016).

Moderate (multifocal) necrosis was shown in mice without probiotic treatment, whereas in probiotic treatment there was no necrosis. Necrosis is a cell death process that occurs in living organisms caused by pathological conditions. Necrosis cell death is caused by acute and irreversible cell damage and cells cannot carry out metabolism, which is caused by the presence of toxins that enter along with the blood flow to the organs (Arcy 2019). Microscopically in necrotic cells, cell boundaries become unclear or disappear. Moderate inflammation was shown in mice without probiotic treatment, while in probiotic treatment there was mild inflammation and the highest concentration of *L. buchneri* E3 (X5) did not show any inflammation. Inflammation is the body's attempt to inactivate or destroy invading organisms, eliminate irritants, and prepare steps to repair tissue structure and function disorders caused by invading organisms (Chen et al. 2017).

REFERENCES

Akin-Osanaiye, Nok AJ, Amlabu E, Haruna E. 2015. Assessment of changed in serum haematological parameters in the the Plasmodium berghei infected albino mice treated with neem (*Azadirachta indica*) extracts. Intl J Chem Biomol Sci 1 (3): 148-152.

Aljahdali NH, Sanad YM, Han J, Foley SL. 2020. Current knowledge and perspectives of potential impacts of *Salmonella enterica* on the profile of the gut microbiota. BMC Microbiol 20: 353. DOI:10.1186/s12866-020-02008-x.

Arcy MAD. 2019. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell Biol Intl 43 (6): 582-592. DOI: 10.1002/cbin.11137.

Azad MAK, Sarker M, Wan D. 2018. Immunomodulatory effects of probiotics on cytokine profiles. Biomed Res Intl 2018: 8063647. DOI: 10.1155/2018/8063647.

Bronte V, Pittet MJ. 2013. The spleen in local and systemic regulation of immunity. Immunity 39 (5): 806-818. DOI: 10.1016/j.immuni.2013.10.010.

Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. 2017. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9 (6): 7204-7218. DOI: 10.18632/oncotarget.23208.

Eng SK, Pusparajah P, Ab Mutalib NS, Ser HL, Chan KG, Lee LH. 2015. *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. Front Life Sci 8: 284-93. DOI: 10.1080/21553769.2015.1051243.

Giles-Gómez M, Sandoval GJG, Matus V, Campos QI, Bolívar F, Escalante A. 2016. In vitro and in vivo probiotic assessment of *Leuconostoc mesenteroides* P45 isolated from pulque, a Mexican traditional alcoholic beverage. Springerplus 5 (1): 708. DOI: 10.1186/s40064-016-2370-7.

Grace D. 2015. Food safety in low and middle income countries. Intl J Environ Res Public Health 12 (9): 10490-10507. DOI: 10.3390/ijerph120910490.

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME. 2014. Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11 (8): 506-514. DOI: 10.1038/nrgastro.2014.66.

Hilscher M, Sanchez W. 2016. Congestive hepatopathy. Clin Liver Dis 8 (3): 68-71. DOI: 10.1002/cld.573.

Hirayama D, Iida T, Nakase H. 2017. The phagocytic function of macrophage-enforcing innate immunity and tissue homeostasis. Intl J Mol Sci 19 (1): 92. DOI: 10.3390/ijms19010092.

La Fata G, Weber P, Mohajeri MH. 2018. Probiotics and the gut immune system: indirect regulation. Probiotics Antimicrob Proteins 10 (1): 11-21. DOI: 10.1007/s12602-017-9322-6.

Lewis SM, Williams A, Eisenbarth SC. 2019. Structure and function of the immune system in the spleen. Sci Immunol 4: 3. DOI: 10.1126/sciimmunol.aau6085.

Lochine N, Arena ET, Bomme P. 2015 Apical invasion of intestinal epithelial cells by *Salmonella typhimurium* requires villin to remodel the brush border actin cytoskeleton. Cell Host Microbes 17 (2): 164-177. DOI: 10.1016/j.chom.2014.12.003.

Mare AD, Ciurea CN, Man A, Tudor B, Moldovan V, Decean L, Toma F. 2021. Enteropathogenic *Escherichia coli*—A Summary of the Literature. Gastroenterol Insights 12 (1): 28-40. DOI: 10.3390/gastroent12010004.

Mikulic J, Bioley G, Corthésy B. 2017. SIga-Shigella immune complexes interact with dectin-1 and SIGNR3 to differentially regulate mouse Peyer's Patch and mesenteric lymph node dendritic cell's responsiveness. J Mol Biol 429: 2387-2400. DOI: 10.1016/j.jmb.2017.05.024.

Mohamed KF, Ashgan MH, Mohamed ML. 2010. Influence of probiotics mixture on *Salmonella typhimurium* in mice. Intl J Microbiol Res 1: 50-61.

Monika MT, Gehlot R, Kumari RA, Priyanka SR. 2021. Antimicrobial properties of probiotics. Environ Conserv J 22 (SE): 33-48. DOI: 10.36953/ECJ.2021.SE.2204.

Nicholson LB. 2016. The immune system. Essays Biochem 60 (3): 275-301. DOI : 10.1042/EBC20160017.

Raabe BM, Artwohl JE, Purcell JE, Lovaglio J, Fortman JD. 2011. Effects of weekly blood collection in C57BL/6 mice. J Am Assoc Lab Anim Sci 50 (5): 680-685.

Ramirez GA, Yacoub MR, Ripa M, Mannina D, Cariddi A, Saporiti N, Ciceri F, Castagna A, Colombo G, Dagna L. 2018. Eosinophils from physiology to disease: a comprehensive review. Biomed Res Intl 2018 (6): 1-28. DOI : 10.1155/2018/9095275.

Robinson M, Harmon C, O'Farrelly C. 2016. Liver immunology and its role in inflammation and homeostasis. Cell Mol Immunol 13 (3): 267-76. DOI : 10.1038/cmi.2016.3.

Shah T, Baloch Z, Shah Z, Cui X, Xia X. 2021. The intestinal microbiota: impacts of antibiotics therapy, colonization resistance, and diseases. Intl J Mol Sci 22: 6597. DOI: 10.3390/ijms22126597.

Shokryazdan P, Jahromi MF, Liang JB, Kalavathy R, Sieo CC, Ho YW. 2016. Safety assessment of two new *Lactobacillus* strains as probiotic for human using a rat model. PLOS ONE 11: e0159851. DOI: 10.1371/journal.pone.0159851.

Steinberg RS, Silva LCS, Souza TC, Lima MT, De Oliveira NLG, Vieira LQ, Arantes RME, Miyoshi A, Nicoli JR, Neumann E, Nunes ÁC. 2014. Safety and protective effectiveness of two strains of *Lactobacillus* with probiotic features in an experimental model of salmonellosis. Intl J Environ Res Public Health 11 (9): 8755-8776. DOI : 10.3390/ijerph110908755.

Vieco-Saiz N, Belguesmia Y, Raspoet R, Auclair E, Gancel F, Kempf I, Drider. 2019. Benefits and inputs from lactic acid bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. Front Microbiol 10: 57. DOI: 10.3389/fmicb.2019.00057.

Wulandari E, Yurmiati H, Subroto T, Suradi K. 2020. Quality and probiotic lactic acid bacteria diversity of rabbit meat Bekasam-fermented meat. Food Sci Anim Resour 40 (3): 362-376. DOI: 10.5851/kosfa.2020.e16.