

ARTICLE

# INTRODUCTION OF THE SYNBIOTIC OF LACTIC ACID BACTERIA FROM DANGKE AND INULIN DAHLIA IN MICE INFECTED WITH ENTEROPATHOGENIC Escherichia coli

[Introduksi Sinbiotik Bakteri Asam Laktat Dari Dangke dan Inulin Dahlia Pada Tikus yang Terinfeksi Enteropathogenic Escherichia coli]

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# ABSTRACT

This study investigates the influence of synbiotics, a combination of probiotics (lactic acid bacteria) and prebiotics (inulin from Dahlia tubers), on mice infected with Enteropathogenic Escherichia coli (EPEC) bacteria. The performance of mice, including weight gain, feed consumption, and feed conversion ratio, was used as parameters to understand the effect of synbiotics on EPEC-infected mice. Four groups of mice were observed in this study: the control group (P1), EPEC infection group (P2), synbiotics introduction group (P3), and synbiotics introduction + EPEC infection group (P4). The research findings demonstrate that synbiotics from Dangke lactic acid bacteria and inulin from Dahlia tubers have a positive impact on mice performance and help them in countering EPEC infection. Group P3, which received synbiotics without EPEC infection, showed significantly higher weight gain and feed conversion efficiency compared to groups P1 and P2. The synbiotics contributed to the enhancement of mice growth and feed efficiency. On the other hand, group P4, which received synbiotics and was infected with EPEC, exhibited a lower survival rate compared to the control group (P1) and group P3, despite having a shorter duration of diarrhea. EPEC caused diarrhea in mice, but synbiotics helped reduce the duration of diarrhea by inhibiting the growth and attachment of EPEC in the mice's intestines. These findings indicate that synbiotics of Lactic Acid Bacteria and inulin Dahlia have the potential as an alternative strategy to improve mice growth and intestinal health. However, the positive effect of synbiotics may be diminished when mice are infected with EPEC.

Keywords: EPEC, Dangke, diarrhea, inulin, lactic acid bacteria

#### ABSTRAK

Studi ini menginvestigasi pengaruh sinbiotik, kombinasi probiotik (BAL asal Dangke) dan prebiotik (inulin umbi Dahlia), terhadap mencit yang diinfeksi dengan bakteri Enteropathogenic Escherichia coli (EPEC). Performa mencit meliputi pertambahan bobot badan, konsumsi pakan, dan konversi pakan digunakan sebagai parameter untuk memahami pengaruh sinbiotik pada mencit yang diinfeksi dengan EPEC. Dalam penelitian ini, empat kelompok mencit diamati, yaitu kelompok kontrol (P1), infeksi EPEC (P2), introduksi sinbiotik (P3), dan introduksi sinbiotik + infeksi EPEC (P4). Hasil penelitian menunjukkan bahwa sinbiotik BAL asal dangke dan inulin umbi Dahlia memberikan dampak positif pada performa mencit dan membantunya menghadapi infeksi EPEC. Kelompok P3, yang diberi sinbiotik tanpa infeksi EPEC, menunjukkan pertambahan bobot badan dan efisiensi konversi pakan yang signifikan lebih tinggi daripada kelompok P1 dan P2. Sinbiotik BAL asal Dangke dan inulin umbi Dahlia mampu meningkatkan pertumbuhan mencit dan efisiensi pakan. Di sisi lain, kelompok P4, yang diberi sinbiotik dan diinfeksi dengan EPEC, menunjukkan tingkat kelangsungan hidup yang lebih rendah dibandingkan kelompok kontrol dan P3, meskipun durasi diare lebih pendek. EPEC menyebabkan diare pada mencit, tetapi sinbiotik dapat membantu mengurangi durasi diare dengan menghambat pertumbuhan dan pelekatan EPEC pada usus mencit. Temuan ini menunjukkan bahwa sinbiotik BAL asal Dangke dan inulin umbi Dahlia memiliki potensi sebagai strategi alternatif untuk meningkatkan pertumbuhan dan kesehatan usus mencit. Namun, efek positif sinbiotik dapat berkurang ketika mencit diinfeksi dengan EPEC.

Kata kunci: Bakteri Asam Laktat, Dangke, diare, EPEC, inulin

#### INTRODUCTION

Diarrhea is one of the common health problems that occur around the world from time to time. The disease causes indigestion characterized by increased stool frequency, a waterier stool consistency, and sometimes accompanied by other symptoms such as nausea, vomiting, and fever. *Escherichia coli* (*E. coli*) is one of the most common microorganisms associated with gastrointestinal infections and diarrhea in humans and animals (Wang *et al.*, 2018a)

Synbiotics are a combination of prebiotics and probiotics. Prebiotics are non-digestible compounds that selectively stimulate the growth and activity of beneficial bacteria in the gastrointestinal tract, while probiotics are live microorganisms that provide health benefits to their hosts. One example of a widely researched prebiotic is inulin, which can be found in dahlia tubers (Febriani, 2022). Lactic acid bacteria (LAB) are a type of probiotic commonly used in food introductions.

One of the unique strains of the lactic acid bacteria group is lactic acid bacteria from Dangke, which is a traditional Indonesian dairy product, especially in the Enrekang Regency of South Sulawesi. Dangke is a type of cheese made from buffalo or cow's milk coagulated with papaya sap. Lactic acid bacteria of Dangke origin have been found to have strong probiotic properties and have the potential to be used in introductions to improve gut health (Hafsan, 2020; Shah *et al.*, 2017; Zachariah, *et al.*, 2019).

Several previous studies have reported a positive effect of lactic acid bacteria introduction on gut health in animals and humans. For example, research on mice conducted by Wang *et al.* (2018) showed that introduction with LAB *Lactobacillus reuteri* HCM2 potentially attenuates the effect of ETEC on colonic microbiota in infected mice. Similar results were also reported in research conducted by Chen *et al.* (2010) which showed that the introduction of this probiotic reduced the severity of diarrhea and length of hospital stay in children with acute diarrhea.

Another empirical evidence supporting the use of probiotics in treating diarrhea and gastrointestinal infections is research by Chen *et al.* (2010) which showed that the introduction of probiotics can reduce diarrhea symptoms and improve children's intestinal health status. A review by Bao & Wu (2021) explained that a diet with a combination of probiotics or other functional foods with a positive impact on gastrointestinal homeostasis can thus shorten the duration of rotavirus diarrhea in children.

In addition, several human studies have also shown the positive effects of lactic acid bacteria introduction on gut health. For example, Servin (2014) revealed that *lactobacilli* and *bifidobacteria*, which inhabit the gastrointestinal microbiota, develop an antimicrobial activity that participates in

the host's gastrointestinal defense system. Another study conducted by Wang *et al.*, (2018) showed that the introduction of lactic acid bacteria can help reduce symptoms of Irritable Bowel Syndrome (IBS) in test animals.

Although there have been many studies that provide promising evidence on the positive effect of lactic acid bacteria introduction on intestinal health, where LAB from Dangke is proven to be able to live well in stomach acidity, has resistance to bile salts (Adawiah, 2014) and can suppress the growth of pathogenic bacteria through invitro testing (Indriani, 2014; Nur et al., 2015). Therefore, this study aims to investigate the effect of the introduction of lactic acid bacteria from Dangke and synbiotics on the diarrhea profile of mice infected with E. coli. This study is expected to complement other similar studies in the use of probiotics and other synbiotics in the treatment of diarrhea, for example, a study by (Yazar et al., 2016) which showed that synbiotic supplementation can reduce the duration of diarrhea in children with acute infectious diarrhea. Similarly, a study by (Rajagukguk et al., 2013) revealed that probiotics and synbiotics have an effect in shortening the duration and frequency of acute diarrhea in children. By supplementing prior research, this study is expected to provide deeper insights into the effectiveness the synbiotics of lactic acid bacteria from Dangke and inulin dahlia tubers in overcoming diarrhea caused by E. coli infection through analysis of diarrhea profiles and survival of mice. The results of this study can potentially influence clinical practice and the development of new products in the form of supplements and new food products containing synbiotics that can be used in efforts to prevent and treat diarrhea and pathogenic bacterial infections in humans and animals.

### MATERIALS AND METHODS

This research was conducted with an experimental approach using Complete Randomized Design. This study aims to determine the effect of LAB synbiotic introduction from Dangke and inulin from Dahlia tubers on the performance and profile of diarrhea experienced by EPEC infected mice with several stages. The materials used in this study include Lactobacillus fermentum from Dangke products isolated and collected by the Microbiology Laboratory of Hasanuddin University, Makassar, Indonesia, as well as inulin from Dahlia tubers produced by PT. Inbio Tech, Jakarta, Indonesia. ICR (Institute of Cancer Research) male mice with an average body weight of 18-33 grams and aged 3 months were obtained from BioLASCO Taiwan Co., Ltd, Taipei, Taiwan. For infection, used Enteropathogenic Escherichia coli (EPEC), strain O127, obtained from ATCC (American Type Culture Collection), Manassas, VA, USA. In addition, mice are given standard feed produced by PT. Charoen Pokphand Indonesia, Jakarta, Indonesia. All stages of this research have received approval Health Committee of UIN with from the Ethics Alauddin letter number B.54.2/KEPK/FKIK/IX/2022.

### Synbiotics preparation of LAB from Dangke and inulin Dahlia

The LAB Those used in the study were aseptically rejuvenated. Pure cultures of bacteria are taken using ose and etched on MRS agar media (de Man, Rogosa, and Sharpe), which consists of peptone, meat extract, yeast extract, glucose, sodium acetate, and other components necessary for bacterial growth. The culture is then incubated at room temperature for 48 hours. After incubation, a suspension with a density of 0.5 McFarland is prepared from cultures that have grown, to ensure a uniform concentration of bacteria.

Synbiotics are prepared by homogenizing 500 mL of fresh cow's milk with the addition of 25 grams of powdered skimmed milk and 50 grams of inulin flour from Dahlia tubers. The mixture is pasteurized by heating and then cooled to a temperature of 40°C. After cooling, a suspension with a density of 109 CFU (Colony Forming Units) of BAL bacteria from Dangke per milliliter, then added as much as 25 mL to the pasteurization mixture. This synbiotic mixture was then homogenized and incubated at 37°C under anaerobic conditions. Anaerobic conditions are achieved using the candle jar technique, in which candles are burned inside a sealed jar to consume oxygen and create a carbon dioxide-rich atmosphere. Culture is placed in a jar before tightly closing (Abdelqader *et al.*, 2013; Derriche *et al.*, 2021).

### **Preparation of experimental animals**

Healthy male mice ICR (strain from Institute of Cancer Research) with an average body weight of 18-33 grams aged 3 months are adapted first. The mice were divided into four treatment groups, each consisting of five mice, the total number of samples was 4 groups x 5 mice = 20 mice. Group P1 is mice that are only given aquadest during treatment, group P2 is mice infected without synbiotics introduction, group P3 is mice that are given synbiotics without EPEC infection, and group P4 is mice that are given synbiotics + EPEC infection. During the experiment, which was 21 days, all groups of mice were given standard feed by adlibitum. The group that received EPEC infection treatment was intervened on day 10. The synbiotics quality control used was carried out by ensuring that the concentrations of inulin and BAL were consistent within the synbiotics suspensions given to the P3 and P4 treatment groups. Synbiotics suspension is given with a content of 0.3 ml/kg BB, and EPEC suspension given to each mouse is 0.2 mL/kg of weight (10<sup>6</sup> CFU/mL suspensi). All stages of treatment of test animals have been approved by the Health Assessment Ethics Committee of UIN Alauddin with letter No. B.54.2/KEPK/FKIK/IX/2022.

### Growth and feed consumption of mice

Body weight measurements were taken at the start of the trial before treatment was given and also at three-day intervals during the 21-day treatment. Feed consumption is determined by calculating the amount of feed consumed by each Mice measured at time intervals every three days using a digital scale. Feed consumption is calculated by subtracting the weight of the remaining feed from the amount of feed originally given *Feed Consumtion* = *Number of starting feed* – *Remaining Feed*. The FCR (Feed Conversion Ratio.) value is calculated by dividing the total feed consumption by the weight gain of mice.

### **Observation of diarrhea Survival of Mice**

The occurrence of diarrhea in experimental mice is observed through physical observation by paying direct attention to whether or not the feces are slimy by looking at the shape of the feces (solid, semi-solid, liquid). This diarrhea observation was done by changing the cage mat in the form of husks into HVS paper and then observing the consistency of feces. If the paper around the feces absorbs more water than normal feces, the animal is certainly experiencing diarrhea (Rachmawati, 2016).

The survivability of mice was determined by looking at the number of mice that survived until the last day of observation (day 21). The calculation of the percentage of mice that survive is by dividing the number of mice that live during a certain time interval by the initial population, multiplied by 100% (Rachmawati, 2016).

# RESULTS

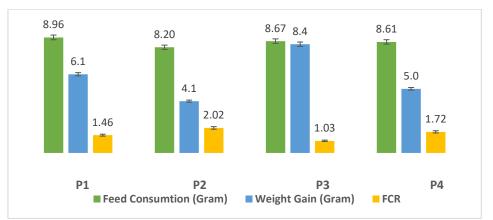
This study focused on the effect of synbiotics on mice infected with Escherichia coli bacteria. Synbiotics are a combination of probiotics (lactic acid bacteria) and prebiotics (inulin dahlia) that work synergistically to improve the health of the digestive system and promote the growth of living things, including mice. The performance of mice (weight gain, feed conversion, diarrhea profile and survival of mice became parameters to understand the effect of the synbiotics introduction of mice infected with EPEC.

### Weight gain and feed efficiency of mice

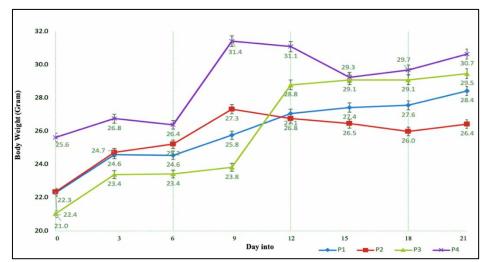
The performance of mice in the treatment group observed in this study was weight gain, feed consumption, and feed conversion. Determination of body weight gain, feed consumption, and feed conversion was carried out on treated mice for 21 days with adlibitum feeding and uniform maintenance standards for the entire group. Based on the results of measurements carried out along with the results of data analysis, there were very significant differences in body weight gain, cumulative feed consumption and Mice feed conversion values between treatment groups (P < 0.001)

as described in Figure 1. The growth of mice was observed through changes in Mice body weight every three days for 21 days as described in Figure 2.

Kelompok kontrol negatif (P1) yang hanya diberikan aquadest menunjukkan peningkatan berat badan rata-rata sebesar 6.1 gram dan konsumsi pakan kumulatif sebesar 8.96 gram, dengan nilai konversi pakan sebesar 1.46. Kelompok yang diinfeksi EPEC tanpa pengenalan sinbiotik (P2) menunjukkan penambahan berat badan rata-rata yang lebih rendah sebesar 4.1 gram dan konsumsi pakan kumulatif yang relatif tinggi sebesar 8.20 gram, sehingga nilai konversi pakannya lebih tinggi sebesar 2.02, menunjukkan penurunan efisiensi pakan. Sebaliknya, kelompok yang diberikan sinbiotik tanpa infeksi EPEC (P3) menunjukkan peningkatan berat badan sebesar 8.4 gram dan konsumsi pakan kumulatif hampir menyerupai kontrol negatif sebesar 8.67 gram, menghasilkan nilai konversi pakan yang rendah, yaitu 1.03, menunjukkan peningkatan efisiensi konversi pakan. Kelompok yang diberikan sinbiotik dan diinfeksi EPEC (P4) menunjukkan peningkatan berat badan yang lebih tinggi dibanding P3 dan kontrol negatif, sebesar 5.0 gram, meskipun sedikit lebih tinggi dibandingkan P2, dengan konsumsi pakan kumulatif yang relatif tinggi pakan kumulatif yang relatif tinggi yaitu 8.61 gram dan nilai konversi pakan sebesar 1.72.



**Figure 1.** Mice Feed consumption, weight gain and feed conversion (FCR) of P1 (aquadest), P2 (aquadest + EPEC infection), P3 (synbiotics introduction), and P4 (synbiotics introduction + EPEC infection) for 21 days of treatment). (*Konsumsi pakan tikus, penambahan berat badan dan konversi pakan (FCR) P1 (aquadest), P2 (aquadest + infeksi EPEC), P3 (pengenalan sinbiotik), dan P4 (pengenalan sinbiotik + infeksi EPEC) selama 21 hari perlakuan*)



**Figure 2.** Mice Body Weight of P1 (aquadest), P2 (aquadest + EPEC infection), P3 (Synbiotics introduction), and P4 (synbiotics introduction + EPEC infection) for 21 days of treatment.). (*Berat Badan Tikus P1 (aquadest), P2 (aquadest + infeksi EPEC), P3 (pengenalan sinbiotik), dan P4 (pengenalan sinbiotik + infeksi EPEC) selama 21 hari perlakuan*).

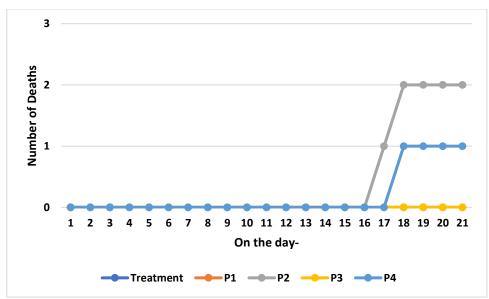
# Profile of diarrhea incidence Survival of mice

Observation of diarrhea carried out refers to the process of observing and recording the time and period of diarrhea experienced by mice. Diarrhea in mice is defined as a condition of defecation that is watery and of higher frequency than usual. Observation of diarrhea in mice based on direct observation of the consistency of feces (solid, mushy, or watery) and observing the absorption of fecal water on HVS paper then comparing with normal feces of mice. The results of observations of diarrhea incidence in mice in all treatment groups as in Table 1.

**Table 1.** Diarrhea Profile of Mice of the treatment group after Infection with Enteropathogenic Escherichia coli (EPEC) on Day 10 (*Profil Diare Tikus dari kelompok perlakuan setelah Infeksi Enteropathogenic Escherichia (EPEC) pada Hari ke-10*)

<b>Treatment</b> (Perlakuan)	<b>Diarrhea occurs on the day</b> (Diare terjadi pada hari)	Period of Diarrhea (Masa diare) (Day)
P1	-	0
P2	13	6
P3	-	0
P4	17	2

Mice survival refers to the ability of mice to survive during experiments. Survival measurement in a study to understand the effect of four treatment groups on Mice survival. The death of mice is used as a reference to measure their survival. Figure 2 outlines the number of mice that died and the percentage of survival of mice after 21 days in each treatment group. The percentage of survival of mice after infection with EPEC is determined by dividing the number of surviving mice by the total number of mice at the start of treatment. The P1 and P3 groups showed 100% survival, while in the groups infected with EPEC, namely P2 and P4, there were mice who died after exposure to diarrhea so that the survival percentage was 60% and 80% respectively.



**Figure 3.** Mice survival of P1 (aquadest), P2 (aquadest + EPEC infection), P3 (Synbiotics introduction), and P4 (synbiotics introduction + EPEC infection) for 21 days of treatment). (*Kelangsungan hidup tikus P1 (aquadest), P2 (aquadest + infeksi EPEC), P3 (pengenalan sinbiotik), dan P4 (pengenalan sinbiotik + infeksi EPEC) selama 21 hari perlakuan*).

## DISCUSSION

### Weight gain and feed efficiency of mice

Based on the results of data analysis, the three performance parameters of mice which include body weight gain, feed consumption and feed conversion value showed a very significant difference (P < 0.01). This indicates that the growth of mice is influenced by the introduction of treatment of synbiotics and EPEC bacteria.

The P1 group was a control group that was only given aquadest without synbiotics or EPEC infection showing a weight gain of 6.1 with an FCR of 0.48. The P2 group was a group infected with EPEC without being given synbiotics showing lower body weight gain, this result indicates a negative effect of EPEC infection on Mice growth and feeds conversion efficiency.

The P3 group is a group introduced to synbiotics. The results showed that mice in this group showed a high weight gain of 8.4, and FCR of 0.31. Thus, the introduction of synbiotics in normal mice increases feed conversion efficiency and leads to higher weight gain. Based on the graph in Figure 4.2 and the results of data analysis, it was proven that the high weight gain of mice in the P3 (Synbiotics) group showed that synbiotics supplementation of lactic acid bacteria from Dangke and Inulin Dahlia tubers positively affected the growth of mice. Feed conversion in the P3 group was lower than in all other groups. This indicates that synbiotics can increase the efficiency of feed conversion into growth.

The P4 group was given synbiotics supplementation and infected with EPEC. Despite synbiotics supplementation, EPEC infection appeared to have a negative influence on Mice growth in this group. The weight gain of this group was lower than that of the P3 group, but still higher than that of the P2 group (EPEC infection). This indicates that synbiotics administration may provide partial protection against the negative effects of EPEC infection on Mice growth.

Synbiotics supplementation of LAB from Dangke and Inulin Dahlia tubers has positive potential in increasing Mice growth and feeding efficiency in Mice. Synbiotics can increase weight gain and feed conversion efficiency, which is an indicator of animal health and productivity. Synbiotics has the potential to improve weight growth and feed conversion efficiency in animals through synergies between prebiotics and probiotics. Although this concept is promising, empirical evidence supporting this synergistic influence still needs to be strengthened with continued research. The synergy between prebiotics and probiotics is expected to improve gut health and microbiota balance, which could theoretically improve nutrient absorption, inhibit pathogen growth, and modulate immune responses in animals. However, the final effect of synbiotics on growth performance and animal health still needs to be established through more extensive and detailed research.

Synbiotics contain probiotics, which are lactic acid bacteria such as LAB from Dangke, which are living microorganisms that are beneficial to the body. When consumed, these probiotics can form colonies in the gut and improve the balance of gut microbiota. This helps prevent the growth of harmful pathogens and ensures the survival of microorganisms that aid digestion (Markowiak &; Śliżewska, 2017). One of the relevant studies is a study conducted by Huang *et al.*, (2023) who applied synbiotics to mice to understand their effects on gut microbiota. The results showed that synbiotics supplementation *of Clostridium butyricum* and chitooligosaccharides was able to modulate the abundance and composition of gut microbiota. This suggests that synbiotics play a role in modulating gut microbiota composition and creating a healthier environment (Valcheva *et al.*, 2019).

Probiotics in synbiotics also produce organic acids such as lactic acid. These acids lower the intestinal pH, creating a less favorable environment for pathogen growth. In addition, the acidic environment helps in the absorption of minerals that are essential for animal growth. These organic acids help lower gut pH and create a less favorable environment for the growth of pathogenic bacteria such as *E. coli*. The production of these organic acids also plays a role in increasing the absorption of certain minerals, which contributes to the increased growth of test animals (Derriche *et al.*, 2021; Kavas *et al.*, 2022).

Prebiotics in synbiotics, such as Dahlia tuber inulin, serve as food for probiotics and other beneficial bacteria in the gut. This leads to better growth and activity of beneficial bacteria, thereby

improving the capacity of the intestines to absorb nutrients from food. A study by Huang, *et al.* (2020) evaluated the effect of synbiotics on mice associated with increased nutrient absorption. The results showed that synbiotics can improve the ability of the Mice intestines to absorb nutrients, especially calcium and magnesium. This can have a positive impact on the growth and development of animals, as well as the efficiency of feed conversion.

Findings from this study suggest that the synbiotics LAB origin Dangke and Inulin Tuber Dahlia have the potential as alternative strategies to improve gut health and animal growth. However, further research is needed to understand the mechanism of action of synbiotics and how the synergy between prebiotics and probiotics can be more optimal in dealing with pathogenic conditions such as EPEC.

## Profile of diarrhea incidence Survival of mice

Group P1 was a control group that was only given aqueous water without any other treatment. The results showed that none of the mice had diarrhea until the last day of observation. In addition, the survival rate reaches 100%. These results showed that giving pure water did not cause diarrhea or death in mice.

The P2 group is a group of mice given aqueous and infected with EPEC, experiencing diarrhea starting from the 13<sup>th</sup> day with a duration of diarrhea for 6 days. Although diarrhea occurs in some mice, the survival rate reaches 80%. This indicates that EPEC administration causes diarrhea in mice and affects survival rates.

Group P3 is a group that is given synbiotics. The observations showed that none of the mice had diarrhea and the survival rate reached 100% until the last day of observation. This suggests that synbiotics administration does not cause diarrhea or death in mice, and mice in this group have optimal survival rates.

The P4 group was introduced to synbiotics and also infected with EPEC. The results showed that mice in this group experienced diarrhea on the 17th day for only 2 days. Survival reaches 80%. These results suggest that synbiotics administration before EPEC infection can reduce the duration of diarrhea, but survival rates are still decreased compared to the control group (P1) and synbiotics group (P3). These results indicate that EPEC administration causes diarrhea in mice, but survival rates remain relatively high. Synbiotics administration does not cause diarrhea or death in mice and may even reduce the duration of diarrhea in mice infected with EPEC. Despite this, the use of synbiotics did not fully restore survival rates to those of the control group (P1).

EPEC bacteria are pathogenic strains of *Escherichia coli* bacteria that can cause infections in the gastrointestinal tract. When mice are given EPEC, these bacteria interact with intestinal epithelial cells, which can cause changes in bowel function and cause diarrhea. Such bacteria are usually attached to the surface of intestinal epithelial cells and form cytoplasm that causes changes in the structure and functionality of the cell. It interferes with the absorption of nutrients and water, causing increased secretion of water and electrolytes, thus eventually causing diarrhea in mice (Galdeano *et al.*, 2019).

Synbiotics administration aims to increase the population of beneficial microorganisms in the intestine and restore the balance of intestinal microflora (Kim *et al.*, 2019). Synbiotics can help inhibit the growth and attachment of pathogenic bacteria such as EPEC to the gut. Probiotics can compete with pathogenic bacteria for a place attached to intestinal epithelial cells, as well as secrete antimicrobial compounds that inhibit the growth of pathogenic bacteria (Servin, 2004). In addition, prebiotics in synbiotics provides nutrients to beneficial bacteria, thus aiding their growth and activity (Markowiak &; Śliżewska, 2017).

Overall, EPEC administration causes diarrhea in mice, while synbiotics administration can help reduce the duration of diarrhea and have a protective effect on the intestines of EPEC-infected mice. However, it is possible that synbiotics have not fully restored the survival rate of mice to the same level as the control group. The mechanism of interaction between EPEC and synbiotics involves various biological processes occurring within the gastrointestinal tract. Synbiotics, which are a combination of prebiotics and probiotics, interact with EPEC at various levels to influence the growth and activity of these bacteria, as well as respond to the body's immune system to infection (Bao &; Wu, 2021; Kemgang *et al.*, 2014; Galdeano *et al.*, 2019; Martínez-Augustin *et al.*, 2014).

The possible interaction is through growth inhibition and EPE C attachment. This mechanism may include the production of antimicrobial compounds such as lactic acid, bacteriocin, and other compounds that can inhibit EPEC growth. In addition, probiotics also compete with EPEC to attach to intestinal epithelial cells, reducing EPEC's chances of colonizing and causing infection. A study conducted by Zhang *et al.* (2018) in Pigs found that synbiotics supplementation containing lactic acid bacteria (BAL) and inulin prebiotics significantly reduced the amount of *E. coli* bound to the intestinal surface of mice. This inhibition of adhesion is likely due to competition between probiotic bacteria and *E. coli* for a place on the surface of the intestinal epithelium, thereby inhibiting the growth and activity of *E. coli*.

Another mechanism is through increasing intestinal epithelial resistance. Synbiotics, especially prebiotics, can provide nutrients for beneficial bacteria in the gut, which then produce Short-Chain Fatty Acids (SCFAs), such as acetic, propionic, and butyric acids. SCFAs can strengthen the intestinal mucosal lining and increase the strength and resilience of intestinal epithelial cells. Thus, intestinal epithelial resistance increases and the likelihood of EPEC attaching to epithelial cells becomes lower (Wang *et al.*, 2019) Another study conducted by Yu *et al.*, (2020) on Broilers found that synbiotics supplementation can increase intestinal epithelial resistance through immune response modulation mechanisms and increased protective mucus production. The results showed that chickens fed with synbiotics had better mucosal thickness and higher quality of intestinal epithelium, which contributed to better protection against pathogenic infections.

Modulation of the body's immune response is also one of the interaction mechanisms between BAL and inulin Dahlia tubers that support the immune system. The mechanism stimulates the production of immune cells, such as T cells and B cells, as well as the regulation of the production of cytokines and anti-inflammatory substances. Synbiotics can also help reduce inflammation caused by EPEC infection. It helps maintain the microbalance of intestinal flora and protects intestinal epithelial cells from damage caused by excessive inflammation (Dinan & Cryan, 2017). A study by (Chen *et al.*, 2010) found that synbiotics containing lactic acid bacteria and prebiotics can increase the production of the anti-inflammatory cytokine interleukin-10 (IL-10) and reduce the production of the pro-inflammatory cytokine interleukin-6 (IL-6). This suggests that synbiotics have the potential to reduce inflammation in the gut and affect the body's immune response to infection.

# CONCLUSION

This study concludes that the introduction of synbiotics in the form of a combination of lactic acid bacteria from Dangke and Inulin Tuber Dahlia has a positive effect on the growth and efficiency of feed consumption of mice infected with EPEC. In addition, the introduction of synbiotics can reduce the risk of diarrhea due to EPEC infection and maintain the survival rate of mice. Synbiotics help maintain the microbalance of the intestinal flora and increase the resistance of intestinal epithelial cells, thus protecting mice from digestive disorders caused by EPEC. However, the survival rate of mice can still be affected by a variety of other factors, including an individual's immune response and the severity of the infection.

# AUTHOR CONTRIBUTIONS

FN: Create research concepts, drafting the article; NH: investigation and collecting research data; H: Create research concept, Data validation and analyze, revise manuscripts; CM: final revision of manuscript; FK: final revision of manuscript.

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