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Original Article

Probiotic *Weisella parameseteroides* on enteropathogenic *E. coli*-induced diarrhea

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Abstract

Background Enteropathogenic *Escherichia coli* (EPEC) is a causative agent of intestinal inflammation and microfloral imbalance, leading to diarrhea. The presence of tumor necrosis factor-α (TNF-α) in the feces is an indicator of inflammation in the intestinal mucosa. *Dadih,* (local made of fermented buffalo milk), contains probiotics and is widely consumed by the people in West Sumatera, Indonesia. *Weisella parameseteroides*, a probiotic lactic acid bacteria (LAB), has been isolated from *dadih* and is believed to be useful for improving intestinal microflora balance and inhibiting the activity of harmful microbes.

Objective To determine the efficacy of *W. parameseteroides* administration in various doses and durations on bowel frequency, stool's TNF-α levels, and intestinal microflora balance on mice with EPEC-induced diarrhea.

Method This randomized experimental animal study examined two factors relating to the effects of *W. parameseteroides* on EPEC-induced diarrhea, namely doses of probiotics (factor A), and durations of observation (factor B). The subjects consisted of 100 male white mice (*Mus musculus*) aged 8 weeks, with weights of 25-30 grams. The outcomes measured were bowel frequency, stool's TNF-α levels, and the balance of intestinal microflora on mice with EPEC-induced diarrhea. Subjects were divided into 5 groups: the negative control group (received neither EPEC nor probiotic), positive control group (received only EPEC), and three experimental groups (received EPEC and different doses of *W. parameseteroides*). Probiotics were given twice at the 12-hours and 24-hours for the experimental groups, while the durations of observation consisted of baseline, 12 hours, 24 hours, and 36 hours.

Results After 36 hours, subjects with EPEC-induced diarrhea who received *W. parameseteroides* administration in doses of $2x10^8$ (A3), were found to have the largest decline of mean defecation (a 4.4-fold decline) and the largest decline of stool's mean TNF-α levels (48.3 pg/mL), compared to the positive control group, and other experimental groups who received higher doses of probiotics. The highest increase of mean LAB (up to 57.50×10^7 cfu/g),the lowest mean of aerobic bacteria (2.5×10^7 cfu/g), and *E. coli* (1.5×10^7 cfu/g) were also found in A3 group.

Conclusion Administration of *W. parameseteroides* at the dose of $2x10^8$ has beneficial effects on reducing bowel frequency, decreasing stool's TNF-α levels, and improving the balance of intestinal microflora in mice EPEC-induced diarrhea. **[Paediatr Indones. 2014;54:1-8.]**

Keywords*: Weisella parameseteroides, TNF-α, diarrhea, EPEC, intestinal microflora*

Diarrhea is a major cause of child mortality worldwide.[1] Annually as many as 6 million children die from diarrhea, with most deaths occurring in developing countries.[2] The two most common causes of diarrhea are viruses and bacteria. *Enteropathogenic Escherichia coli* (EPEC) in concentration of 10^5 - 10^{10} cfu/mL have been shown to cause diarrhea.[3] EPEC adhesion

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to intestinal mucosal cells leads to changes of cell structure, followed by bacterial invasion into the intestinal epithelial cells.[4]

The occurrence of inflammation of the bowel mucosa is evidenced by the presence of tumor necrosis factor-α (TNF-α) in feces, and is responsible for intestinal mucosal damage. Tumor necrosis factor-α is a pleotropic cytokines that stimulate inflammation.[5] High TNF-α level will damage the enterocytes tight junctions of intestinal mucosa. The cumulative result of gut atrophy and tight junctions destruction are increased membrane permeability, disrupted intestinal absorption and diarrhea.[6] Acute diarrhea also results in microflora imbalance. The balance of microflora in the digestive system is very important, as infection by bacterial pathogens may cause intestinal microecological changes and colonization resistance of the intestinal mucosa.[7]

Probiotic is a viable bacteria given as a dietary supplement to benefit human health by improving the balance of intestinal microflora. Probiotic bacteria may reduce the occurrence of diarrhea and inhibit the production of proinflammatory cytokines.[8,9] A study on mice given lipopolysaccharide (LPS) showed that TNF-α, which stimulates tissue damages and apoptosis, was inhibited after administration of *Lactobacillus rhamnosus* GG. *Dadih*, a local made of fermented buffalo milk, is a traditional food of West Sumatra, Indonesia, may be classified as a probiotic source, since it is the product of lactic acid bacteria (LAB) fermentation. Lactic acid bacteria are useful in human digestion, a s they are able to inhibit the growth of harmful microbes and bacteria.[10,11] One of probiotic microbes or LAB isolated from *dadih* is *W.paramesenteroid*es,[12] which produces the bacteriocin weisellin that is consisted of 43 amino acids and has anti-bacterial activity.[13] We aimed to determine the effect of various doses and durations of *W.paramesenteroides* administration on bowel frequency, TNF-α level in feces, and the intestinal microflora balance in mice with EPEC-induced diarrhea.

Methods

We conducted a randomized experimental animal study in April 2012 at the Biomedical Laboratory and Laboratory of Technology Animal Husbandry of Andalas University, Padang West Sumatera. Since the similar of total intestinal microflora with human,[14] in this study we sused the male white mice (*Mus musculus*) obtained from the Animal Development Laboratory of the Pharmacy Department, Andalas Universitiy. We used 120 mice, aged 8 weeks, with weights of 25-30 grams.

Mice were randomized into groups to compare the influence of two factors relating to *W. paramesenteroides*: doses of probiotic administration (factor A) and durations of observation (factor B). The groups were classified as follows: a negative control group (A1), given only standard feed and water, a positive contro group (A2), given EPEC at a dose of 10^8 cfu; and three experimental groups that received both EPEC at dose of 10^8 cfu and *W. paramesenteroides* doses of 2 x 10^8 cfu/g (A3), 2 x 10^9 cfu/g (A4), or 2 x 10^{10} cfu/g (A5). The probiotic were given twice at 12 hours and 24-hours for the experimental groups. The second factor (B) was the duration of observation, consisting of 0 hour (B1), 12 hours (B2), 24 hours (B3), and 36 hours (B4) (B2), 24 hours (B3), 36 hours (B4).

The first experimental week comprised of mice acclimatization. During this period, all of 120 mice were given standard food and drink. After the acclimatization period, the mice with average weight of 27 grams were randomly assigned to groups, as shown in **Figure 1**.

Probiotic isolate of *W. paramesenteroides* was obtained from the previouw study,[12] while the bacterial isolate of enteropathogenic *Escherichia coli* was provided by the Faculty of Animal Science, Andalas University.

Diarrhea in mice was defined as the bowel frequency more than twelve times a day[15] or watery stool.[16] We placed plastic sheets on the base of mice cage, so that we could measure the bowel frequency from the stool mark on it after 36 hours. The stool TNF-α level was measured using ELISA kit of *ABO Switzerland®*. The balance of intestinal microflora was measured by counting the colony form unit in bacterial culture. The mice had been sacrificed at each time point using ether before the surgical intestinal tissue sampling, which then cultured in *de Mann Rogosa Sharpe* (MRS) Broth for LAB, *Plate Count Agar* (PCA) for aerob bacteria, and *Mac Conkey Agar* for *E. coli*.

In order to determine the treatment effect and

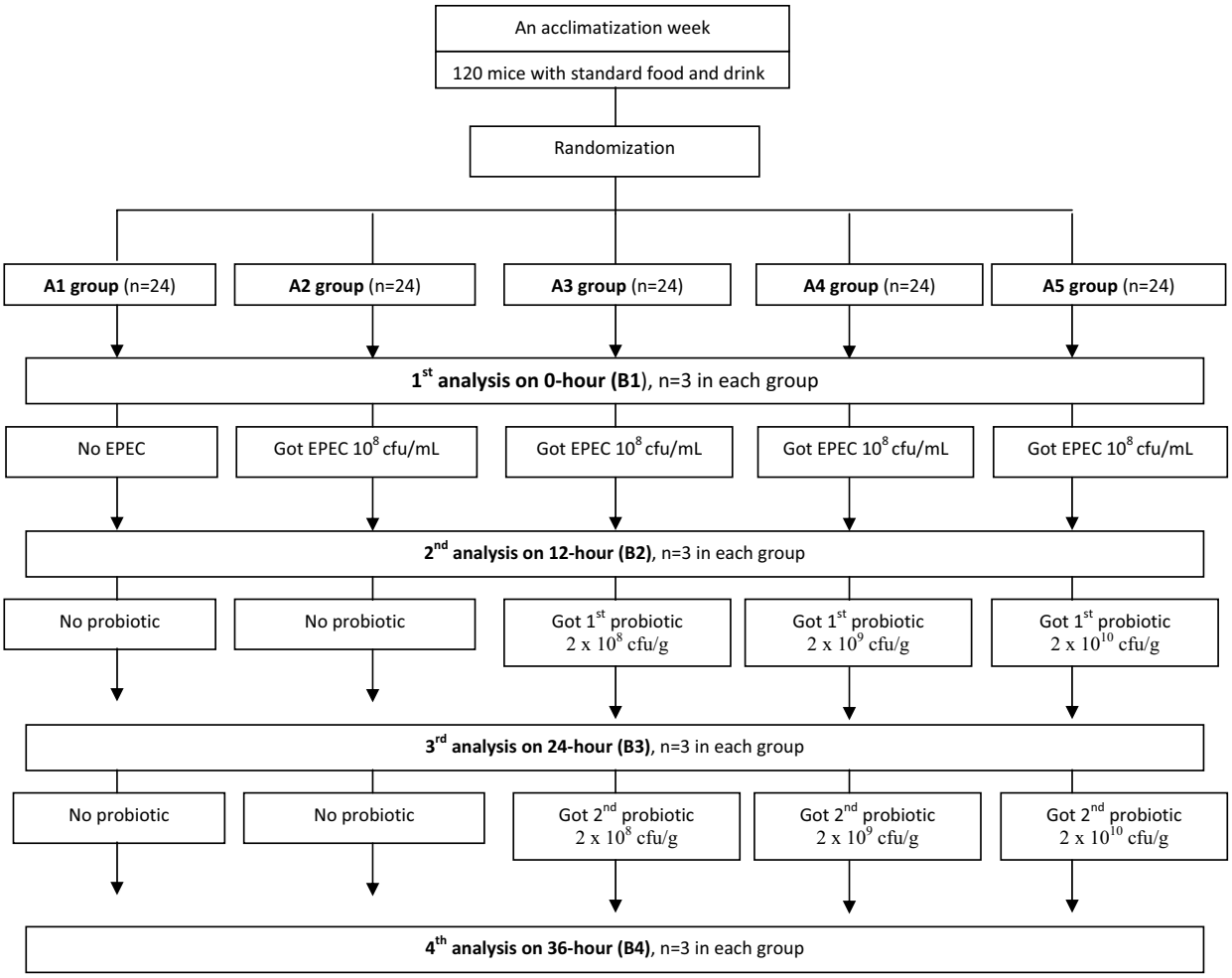

Figure 1. Study flow chart

the interaction of the observed variables we used variance analysis or ANOVA. When treatment had an effect, we continued the analysis with *Duncan's multiple range test* (DMRT).

Results

Statistical analysis revealed a highly significant interaction (P=0.01) between factors A and B on bowel frequency. Mean bowel frequencies for each treatment combination are presented in **Table 1**.

After the acclimatization period mice in all groups had the similar mean of bowel frequency (B1). Increased mean bowel frequency was seen in groups with EPEC administration (A2, A3, A4, A5) compared to the group without EPEC administration (negative control/A1) after 12 hours (B2). The highest mean bowel frequency was in tehe positive control group (50.5), which was a 10-fold increase over the negative control group (5.0).

Decreased mean stool frequency was seen at 36 hours (B4) in groups with EPEC administration. After twice 24 hours of administration *W. paramesenteroides* in doses $2x10^8$, $2x10^9$ and $2x10^{10}$ cfu/g, the experimental groups (A3, A4, A5) had a significant decline compared to the positive control (A2), with $P < 0.01$. The largest decline (point B2 compared to point B4) was found in th experimental group with *W.paramesenteroides* administration in doses of 2×10^8 (A3), which was 4.4-fold decline compared to the A2, A4, and A5 groups (1.4-fold, 1.9-fold, and 2.6-fold, respectively).

Statistical analysis also showed a highly significant interaction ($P < 0.01$) between factor A (dose) and factor B (duration of observation) for TNF-α levels in feces. Mean TNF-α levels for each treatment combination are presented in **Table 2**.

Groups with EPEC administration (A2, A3, A4, A5) had increased stool mean TNF-α levels compared to the group without EPEC administration (negative control/A1) after 12 hours (B2). The highest mean of stool TNF-α levels was in the positive control group (127.7 pg/mL), which was a 10.4-fold increase over the negative control group (12.3 pg/mL).

The mean of stool TNF-α levels were decreased in the groups with EPEC administration (A2, A3, A4, A5) after 36 hours (B4). The experimental groups (A3, A3, A5) had a significant decline compared to the positive control (A2), with $P < 0.01$. The experimental group *W. paramesenteroides* administration in doses of $2x10^8$ (A3) had the largest decline (48.3 pg/mL) compared to the A2, A4, and A5 groups (40.3 pg/mL, 35.4 pg/mL, and 35 pg/mL, respectively).

Statistical analysis showed highly significant interactions ($P < 0.01$) between factor A (doses) and factor B (durations of observation) in mean intestinal microflora of the mice for LAB (**Table 3**), aerobic bacteria (**Table 4**) and *E. coli* (**Table 5**).

Table 3 shows the increase of total mean LAB in mice intestines after twice administration of *W. paramesenteroides*. The highest mean (57.50×10^7 cfu/g) was observed at 36 hours (B4) after administration in the dose of $2x10^8$ cfu/g (A3), a 11-fold higher than the positive control groups (A2).

Table 4 shows the lowest mean aerobic bacteria ($2.5x10^7$ cfu/g) was observed at 36 hours (B4) after administration of $2x10^8$ *W. paramesenteroides* (A3), which was 12.8-fold lower than the positive control

Tabel 1. Mean bowel frequency* based on doses and duration of observation

Factor A (doses)	Factor B (durations of observation)				Total	Mean (SD)
	B1 (0 hour)	B2 (12 hours)	B3 (24 hours)	B4 (36 hours)		
A1 (negative control)	4.0	5.0	4.5	5.0	18.5	4.6 (1.1)
A2 (positive control)	5.0	50.5	41.0	37.0	133.5	33.9 (2.9)
A3 ($2x10^8$ cfu/g)	5.5	48.5	16.5	11.0	81.5	20.4 (2.3)
A4 ($2x10^9$ cfu/g)	5.0	30.5	20.0	16.0	71.5	17.9 (2.1)
A5 ($2x10^{10}$ cfu/g)	5.5	28.5	12.5	11.0	57.5	14.4 (1.9)

*Mean bowel frequency: number of times per 12 hour block time

Table 2. Mean TNF-α levels* in feces based on doses and duration of observation

Factor A (doses)	Factor B (duration of observation)				Total	Mean (SD)
	B1 (0 hour)	B2 (12 hours)	B3 (24 hours)	B4 (36 hours)		
A1 (negative control)	13.2	12.3	12.7	12.2	50.5	12.6 (1.8)
A2 (positive control)	13.3	127.7	87.4	87.4	351.7	87.9 (4.7)
A3 ($2x10^8$ cfu/g)	13.3	63.5	15.2	15.2	129.9	32.5 (2.9)
A4 ($2x\ 10^9$ cfu/g)	12.3	53.8	18.1	18.1	107.3	26.8 (2.7)
A5 ($2x10^{10}$ cfu/g)	11.8	55.6	20.6	20.6	127.7	31.9 (2.8)

* TNF-α levels (pg/mL)

Table 3. Mean LAB* in mice intestines based on doses and durations of observation

Factor A (doses)	Factor B (duration of observation)				Total	Mean (SD)
	B1 (0 hour)	B2 (12 hours)	B3 (24 hours)	B4 (36 hours)		
A1 (negative control)	4.0	4.5	4.0	4.0	16.5	4.1 (1.0)
A2 (positive control)	4.0	6.5	1.5	5.0	17.0	4.3 (1.0)
A3 ($2x10^8$ cfu/g)	5.5	6.5	44.5	57.5	114.0	28.5 (2.7)
A4 ($2x10^9$ cfu/g)	4.5	5.5	53.5	15.5	79.0	19.8 (2.2)
A5 ($2x10^{10}$ cfu/g)	4.5	4.5	53.0	25.5	87.5	21.9 (2.3)

*LAB ($x\ 10^7$ cfu/g)

group (A2). The positive control group (A2) had the highest mean of aerobic bacteria at 24 hours (B3 = 33.0x10^7 cfu/g), a 3-fold higher over the negative control group (A1=10.5x10^7 cfu/g).

Table 5 shows the lowest meatn E. coli (1.5x10^7 cfu/g) was observed at 36 hours (B4) after administration of 2x10^8 *W. paramesenteroides* (A3), which was 8-fold lower than the positive control group (A2).

intestinal epithelial cells. After successful attachment and colonization at the intestinal epithelial cells, probiotics produce and secrete anti-microbial metabolites that may inhibit the growth of intestinal EPEC.[17] A study reported that *W. paramesenteroides* produces bacteriocin, weisellin A, which is active against pathogenic bacteria and protects the intestinal mucosa.[13]

Table 4. Mean aerobic bacteria* in mice intestines b ased on doses and durations of observation

Factor A (doses)	Factor B (duration of observation)				Total	Mean (SD)
	B1 (0 hour)	B2 (12 hours)	B3 (24 hours)	B4 (36 hours)		
A1 (negative control)	10.5	12.0	10.5	13.5	46.5	11.6 (1.7)
A2 (positive control)	11.0	8.0	33.0	32.0	84.0	21.0 (2.3)
A3 (2x10^8 cfu/g)	8.0	9.0	4.0	2.5	23.5	5.9 (1.2)
A4 (2x10^9 cfu/g)	6.5	4.5	13.5	10.5	35.0	8.8 (1.5)
A5 (2x10^{10} cfu/g)	7.5	12.0	9.0	5.0	33.5	8.4 (1.5)

*Mean aerobic bacteria (x10^7 cfu/g)

Table 5. Mean *E. coli* in mice intestine based on doses and durations of observation

Factor A (doses)	Factor B (duration of observation)				Total	Mean (SD)
	B1 (0 hour)	B2 (12 hours)	B3 (24 hours)	B4 (36 hours)		
A1 (negative control)	5.5	6.0	7.0	5.5	24.0	6.0 (1.2)
A2 (positive control)	6.0	20.5	19.5	12.5	58.5	14.6 (1.9)
A3 (2x10^8 cfu/g)	6.5	3.0	2.5	1.5	13.5	3.4 (0.9)
A4 (2x10^9 cfu/g)	4.5	3.0	3.5	2.5	13.5	3.4 (0.9)
A5 (2x10^{10} cfu/g)	6.0	4.5	4.0	3.5	18.0	4.5 (2.0)

*Mean *E. coli* (x10^7cfu/g)

Discussion

Previous study showed the normal bowel frequency in mice was less than 12 times per day. Increased bowel frequency was reported in mice given lipopolysaccharide (LPS) from E. coly serotype 0128, reached 30-40 times/day in mice. The study also reported that a probiotic administration could decrease the bowel frequency.[15] Similar to the study, we found the mean bowel frequency were 39.5 times after 12 hours (B2) of EPED-administration (A2, A3, A4, A5). In the 36 hours (B4), the mean bowel frequency was decrease into 12.7 times after twice administration of *W. paramesenteroides* (A3, A4, A5).

W. paramesenteroides as a probiotics may prevent the translocation of EPEC to intestinal epithelial cells, compete with EPEC for the use of essential nutrients in the gut, as well as multiply and attach to the

Enteropathogenic *E. coli* is the first strain *E. coli* known to cause diarrhea[13] when consumed at doses 10^5-10^{10} cfu/mL.[4] The adhesion of EPEC to intestinal mucosal cells leads to changes in cell structure, such that the bacteria are able to invade the intestinal epithelial cells. Injury to intestinal epithelial cells caused by EPEC attachment leads to disrupted homeostasis of the intestinal mucosa, causing excessive fluid secretion into the intestine, hence leading to profuse diarrhea.[18,19] A study reported that rats had diarrhea in the first day after they were given an EPEC dose of 2x10^8 cfu/mL.[20] However, other study reported that diarrhea in mice appeared only in the second week following exposure to EPEC at a dose of 2x10^6 cfu/mL,[21] or on the seventh day with LAB supplemental, while mice without LAB administration suffered from severe diarrhea.[22]

Following EPEC administration, we found that TNF-α levels in mice stool were significantly higher

($P<0.01$). Mean TNF-α concentration in the negative control mice (A1) was significantly different ($P<0.01$) from the positive control group (A2) after 12 hours EPEC administration (12.3 pg/mL vs. 127.7 pg/mL, respectively). This result showed that there was an EPEC-induced inflammatory process, characterized by elevated TNF-α levels in stool, up to 10-fold increase compared to normal levels. These findings were consistent with research conducted by Hsu *et al.* who found a significant increase in serum levels TNF-α in patients with bacterial gastroenteritis.[23] In this study, the mean TNF-α levels were significantly decreased ($P<0.01$) after *W. paramesenteroides* administration, at three different doses of $2x10^8$, $2x10^9$, and $2x10^{10}$ cfu/g. After 36 hours study (B4), the group with *W. paramesenteroides* administration at a dose of $2x10^8$ cfu/g (A3) had the lowest mean of TNF-α level (15.2 pg/mL), but the groups with higher dose of *W. paramesenteroides* administration (A4 = 2x10 cfu/g; A5 = 2x10 cfu/g) had higher mean of TNF-α levels (18.1 pg/mL and 20.6 pg/mL, respectively). These results showed that *W. paramesenteroides* admininistration could reduce the mean TNF-α levels after EPEC infection, but the three doses showed that increasing dose did not act to decrease the inflammation.

Probiotic supplementation may protect against mucosal epithelial cell damage by *E. coli* exposure and protect cell against further damage by TNF-α and interferon (IFN)-γ.[22] Probiotics are able to down regulate T helper (Th)-1 responses and inhibit the production of proinflammatory cytokines, such as TNF-α, interleukin (IL)-12 and IFN-γ by dendritic cells.9 These results were also consistent with other studies in which (*Lactobacillus rhamnosus* GG) LGG specifically inhibited production of TNF-α and its apoptosis or cytotoxic effects,[8] decreased the concentration of TNF-α in the feces,[24] decreased the serum levels of TNF-α, and stool frequency.[25]

The mean total LAB levels showed significant differences ($P< 0.01$) between dosing groups for each duration of observation with the highest mean ($57.5x10^7$ cfu/g) after 36-hours administration of $2x10^8$ cfu/g *W. paramesenteroides*, a 11-fold higher than the positive control groups (A2). These findings were consistent with a study reported the higher total LAB in mice with probiotic administration, compared to the group without probiotic administration, before EPEC-induced.[21] Other study also showed an increased amount of LAB in feces after probiotic *Lactobacillus plantarum 1B1* administration.[26] Lactic acid bacteria provide positive benefits for health, especially for the balance of gastrointestinal microflora and control of pathogenic bacteria in the digestive tract. Lactic acid bacteria is a group of gram-positive bacteria capable of converting carbohydrates into lactic acid, which may have a bactericidal effect on other bacteria by lowering the pH of the environment to between 3 to 4.5 such that other bacterial growth is inhibited.[27]

The mean total aerobic bacteria in our study increased in the positive control group (A2) after 24 hours (B3) EPEC administration to $33.0x10^7$ cfu/g, an increase of 3 times greater than that of the negative control group ($10.5x10^7$ cfu/g). Our results were consistent with a study reported *dadih*, contained 2,8 x 10^9 cfu/g *Lactobacillus*, was found to increase the number of colonies of *Lactobacillus sp* in the duodenum and ileum of mice.[28] The lack of similar increase in the A3, A4, and A5 groups suggests that *W. paramesenteroides* adherence to the intestinal mucosa may inhibit adherence by other viruses or bacteria, in effect, competing with pathogenic bacteria, thereby preventing their colonization.[29,30]

Mean total aerobic bacteria in our study decreased at 36 hours (B4) after administration of 2 x 10^8 cfu/g *W. paramesenteroides* to $2.50x10^7$ cfu/g, lower than the positive control group after EPEC administration. Similarly, Adolfson *et al.* reported that buttermilk *Lactobacillus sp.* invasion was able to reduce pathogenic bacteria in the gut.[31] Other study also found that some isolates of LAB could inhibit pathogenic microorganisms.[32] A study used 2% curd *L. lactis* mutant bacteriocins demonstrated its ability to inhibit the activity of microbial pathogens such as *Staphylococcus aureus* and *Salmonella typhi*.[33] Another study reported *dadih* containing LAB was beneficial for killing pathogenic bacteria in the gut.[34]

Increased mean total *E. coli* (up to $20.5x10^7$ cfu/g) was found in the positive control mice (A2), significantly ($P < 0.01$), at 12 hours (B2), a 3-fold increase over the negative control group (6.00 x 10^7 cfu/g). Mean total *E. coli* in mice gut was significantly decreased in the groups of mice given $2x10^8$ cfu/g *W. paramesenteroides*, to $1.50x10^7$ cfu/g, an 8-fold decrease compared to that in the positive control. These findings are consistent with a study which found lower levels of ($P<0.05$) *E. coli* in

the cecum mucosa of mice during the second week after EPEC exposure and probiotic *L. 2C12* and *L. plantarum 2B4 acidophilus* administration, than in mice exposed to EPEC illness.[20] Probiotics produce antibacterial such as organic acids, free fatty acids, ammonia, hydrogen peroxide, reuterin, bacteriocins, and hydrogen ions that can prevent and inhibit the growth of pathogenic bacteria.[9] These results indicate that *W. paramesenteroides* was able to inhibit the *E. coli* population in intestinal mucosa.

In conclusion, decrease bowel frequency and stool TNF-α levels are found in mice with EPEC-induced diarrhea, 24 hours after the probiotic *W. paramesenteroides* administration in the dose of $2x10^8$ cfu/g, *W. paramesenteroides* also balances the intestinal microflora in mice with EPEC-induced diarrhea.

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References

1. WHO, UNICEF. Diarrhoea: why children are still dying and what can be done. 2009.
2. Subagyo B, Santosa NB. Diare Akut. In: Juffrie M SS, Oswari H, Arief S, Rosalina I, Mulyani NS, *et al* ed. Buku Ajar Gastroenterologi- Hepatologi. Jakarta: IDAI; 2010. p.87-120.
3. Thapar N. Diarrhoea in children: an interface between developing and developed countries. Lancet. 2004;363:641-53.
4. Kelleher SL, Casas I, Carbajal N, Lonnerdal B. Supplementation of infant formula with the probiotic Lactobacillus reuteri and zinc: impact on enteric infection and nutrition in infant rhesus monkeys. J Pediatr Gastroenterol Nutr. 2002;35:162-8.
5. Karuniawati. Pengaruh suplementasi seng dan probiotik terhadap durasi diare cair anak [tesis]. Semarang: Universitas Diponegoro; 2010.
6. Rosalina I. Efikasi pemberian zinc pada diare. In: Naskah lengkap Konas III Badan Koordinasi Gastroenterologi Anak Indonesia; 2007 December 6-8. Surabaya; 2007. p.250-3.
7. Collado MC, Isolauri E, Salminen S, Sanz Y. The impact of probiotic on gut health. Current Drug Metabolism. 2009;10:68-78.
8. Pena JA, Versalovic J. *Lactobacillus rhamnosus* GG decreases TNF-alpha production in lypopolysaccharide-activated murine macrophages by a contact-independent mechanism. Cellular Microbiol. 2003;5:277-85.
9. Ng SC, Hart AL, Kamm MA, Stagg AJ, Knight SC. Mechanism of action of probiotics: recent advance. Inflamm Bowel Dis. 2009;15:300-10.
10. Goel AJ, Dilbaghi N, Kamboj DV, Sing L. Probiotics: microbial therapy for health modulation. Defence Sci J. 2006;56:513-29.
11. Narayan SS, Jalgaonkar S, Shahani S, Kulkarni UN. Probiotics: current trends in the treatment of diarrhea. Hongkong Med J. 2010;16:213-8.
12. Purwati E, Arif, Rahmadi A. Buku ajar teknologi dadih. Bogor: Cendikia Publ House; 2011. p.80-133.
13. Papagianni M, Papamichael EM. Purification, amino acid sequence and characterization of the class IIa bacteriocin weisellin A, produced by *Weisella paramesenteroides* Dx. Bioresource Tech. 2011;102:6730-4.
14. Routhiau VG, Raibaud P, Dubuquoy C, Moreau MC. Colonization of gnobiotic mice with human gut microflora at birth protect against *E. coli* heat-labile enterotoxin-mediated abrogation of oral tolerance. Pediatr Res. 2003;54:739-46.
15. Liu J, Wan R, Xu X-F, Wang XP, Yang WJ, Xia YF, *et al*. Effect of Lianshu preparation on lipopolysaccharide-induced diarrhea in rats. World J Gastroenterol. 2009;15:2009-15.
16. Baker DG. Natural pathogens of laboratory mice, rats, and rabbits and their effects on research. Clinical Microbiology Review. 1998;11:231-66.
17. Walker WA. Mechanism of action of probiotics. Clin Infect Dis. 2008;46:S87-91.
18. Haq JA, Li HC, Rahman RA. Detection of enteropathogenic *Eschericia coli* (EPEC) by serotyping and cell adhesion assay among children in north-eastern Peninsular Malaysia - a hospital based study. Ibrahim Med Coll J. 2008;2:40-3.
19. Lapointe TK, O'Connor PM, Buret AG. The role of epithelial malfunction in the pathogenesis of enteropathogenic *Escherichia coli*-induced diarrhea. Laboratory Investigation. 2009;89:964-70.
20. Hartati AW. Evaluasi aktivitas anti diare isolat Lactobacillus dari ASI [tesis]. Bogor: Institut Pertanian Bogor; 2008.
21. Arief I, Jenie BSL, Astawan M, Witarto AB. Efektifitas probiotik *Lactobacillus plantarum 2 C12* dan *Lactobacillus acidophilus 2B4* sebagai pencegah diare pada tikus percobaan. Media Peternakan. 2010;33:137-43.
22. Astawan M, Wresdiyati, Arief I, Suhesti E. Gambaran hematologi tikus putih (*Rattus norvenicus*) yang diinfeksi

Eschericia coli enteropatogenik dan diberikan probiotik. Media Peternakan. 2011;34:7-13.
23. Hsu TR, Chen SJ, Wu TC, Chung RL, Tang RB. Tumor necroting factor alpha and interleukin 10 in viral and bacterial gastroenteritis in children. J Chin Med Assoc. 2005;68:250-3.
24. Viljanen M. Effect of probiotics bacteria on symptoms on immunologic responses in infants with atopic dermatitis [dissertation]. Helsinki: Medical Faculty of The University of Helskinsi; 2005.
25. Lomax AR, Calder PC. Probiotics, immune function, infection, and inflamation: a review of the evidence from studies conducted in human. Current Pharmaceutical Design. 2009;15:1428-518.
26. Pertiwi WA. Profil mikroflora feses dan usus tikus putih *(Rattus norwegicus)* dengan konsumsi daging yang difermentasi oleh *Lactobacillus plantarum* [tesis]. Bogor: Insitut Pertanian Bogor; 2008.
27. Kusmiati M. Aktivitas bakteriosin dari bakteri *Leuconostoc mesenteroides Pbac1* pada berbagai media. Makara Kesehatan. 2002;6:1-7.
28. Heriyenni. Kajian peranan dadih susu kerbau serta campuran dadih dan virgin coconut oil terhadap performance mencit uji [tesis]. Padang: Universitas Andalas; 2007.
29. Caicedo RA, Schanler RJ, Li N, Neu J. The developing intestinal ecosystem: implications for the neonate. Pediatr Res. 2005;58:625-8.
30. Guarner F, Garisch J, Eliakim R. World Gastroenterology Organisation practice guidelines: probiotics and prebiotics. Arab Journal of Gastroenterology. 2009;10:33-42.
31. Adolfson O, Meydani SN, Russel RM. Yogurt and gut function. J Clin Nutrition. 2004;80:245-56.
32. Sujaya. Isolasi dan karakterisasi bakteri asam laktat dari susu kuda Sumbawa. J Veterinal. 2008;9:52-9.
33. Melia S, Juliyarsi I. Potensi dadih susu sapi mutan *Lactococcus lactis* dengan kandungan bakteriosin terhadap bakteri patogen [tesis]. Padang: Universitas Andalas; 2007.
34. Surono. Probiotik susu fermentasi dan kesehatan. Jakarta: Yayasan Pengusaha Makanan dan Minuman Seluruh Indonesia (YAPMMI); 2004.