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

Aslinar, Yusri Dianne Jurnalis, Endang Purwati RN, Yorva Sayoeti

**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 paramesenteroides*, 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. paramesenteroides* 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. paramesenteroides* 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. paramesenteroides*). 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. paramesenteroides* administration in doses of 2x10^8 (A3), were found to have the largest decline of mean defecation (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 x 10^7 cfu/g), the lowest mean of aerobic bacteria (2.5 x 10^7 cfu/g), and *E. coli* (1.5 x 10^7 cfu/g) were also found in A3 group.

**Conclusion** Administration of *W. paramesenteroides* 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 paramesenteroides*, TNF-α, diarrhea, EPEC, intestinal microflora

**Diarrhea is a major cause of child mortality worldwide.**

Annually as many as 6 million children die from diarrhea, with most deaths occurring in developing countries. The two most common causes of diarrhea are viruses and bacteria. *Enteropathogenic Escherichia coli* (EPEC) in concentration of 10^9 - 10^10 cfu/mL have been shown to cause diarrhea. EPEC adhesion

From the Department of Child Health, Andalas University Medical School, Padang, Indonesia.

Reprint requests to: Aslinar, Department of Child Health, Andalas University Medical School, Jl. Perintis Kemerdekaan No.1 49, PO BOX 49, Padang, Indonesia. Tel. +62-75131746, Fax. +62-75132838. E-mail: ummihirzi@gmail.com.
Probiotic bacteria may reduce the occurrence of diarrhea and inhibit the production of proinflammatory cytokines. 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, as they are able to inhibit the growth of harmful microbes and bacteria. One of probiotic microbes or LAB isolated from dadih is W. paramesenteroides, which produces the acid and has anti-bacterial activity. 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, in this study we used the male white mice (Mus musculus) obtained from the Animal Development Laboratory of the Pharmacy Department, Andalas University. 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 control group (A2), given EPEC at a dose of 10⁶ cfu; and three experimental groups that received both EPEC at dose of 10⁶ cfu and W. paramesenteroides doses of 2 x 10⁸ cfu/g (A3), 2 x 10⁸ cfu/g (A4), or 2 x 10¹⁰ 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). 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 previous study, 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 or watery stool. 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
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 the experimental group compared to the group without EPEC administration (negative control/A1) after 12 hours (B2). The highest mean bowel frequency was in the 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 2x10^9, 2x10^8, 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 the experimental group with W. paramesenteroides administration in doses of 2 x 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 (12.3 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 x 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

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

<table>
<thead>
<tr>
<th>Factor A (doses)</th>
<th>Factor B (durations of observation)</th>
<th>B1 (0 hour)</th>
<th>B2 (12 hours)</th>
<th>B3 (24 hours)</th>
<th>B4 (36 hours)</th>
<th>Total</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (negative control)</td>
<td></td>
<td>4.0</td>
<td>5.0</td>
<td>4.5</td>
<td>5.0</td>
<td>18.5</td>
<td>4.6 (1.1)</td>
</tr>
<tr>
<td>A2 (positive control)</td>
<td></td>
<td>5.0</td>
<td>50.5</td>
<td>41.0</td>
<td>37.0</td>
<td>133.5</td>
<td>33.9 (2.9)</td>
</tr>
<tr>
<td>A3 (2x10^7 cfu/g)</td>
<td></td>
<td>5.5</td>
<td>48.5</td>
<td>16.5</td>
<td>11.0</td>
<td>81.5</td>
<td>20.4 (2.3)</td>
</tr>
<tr>
<td>A4 (2x10^7 cfu/g)</td>
<td></td>
<td>5.0</td>
<td>30.5</td>
<td>20.0</td>
<td>16.0</td>
<td>71.5</td>
<td>17.9 (2.1)</td>
</tr>
<tr>
<td>A5 (2x10^10 cfu/g)</td>
<td></td>
<td>5.5</td>
<td>28.5</td>
<td>12.5</td>
<td>11.0</td>
<td>57.5</td>
<td>14.4 (1.9)</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Factor A (doses)</th>
<th>Factor B (duration of observation)</th>
<th>B1 (0 hour)</th>
<th>B2 (12 hours)</th>
<th>B3 (24 hours)</th>
<th>B4 (36 hours)</th>
<th>Total</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (negative control)</td>
<td></td>
<td>13.2</td>
<td>12.3</td>
<td>12.7</td>
<td>12.2</td>
<td>50.5</td>
<td>12.6 (1.8)</td>
</tr>
<tr>
<td>A2 (positive control)</td>
<td></td>
<td>13.3</td>
<td>127.7</td>
<td>87.4</td>
<td>87.4</td>
<td>351.7</td>
<td>87.9 (4.7)</td>
</tr>
<tr>
<td>A3 (2x10^7 cfu/g)</td>
<td></td>
<td>13.3</td>
<td>63.5</td>
<td>15.2</td>
<td>15.2</td>
<td>129.9</td>
<td>32.5 (2.9)</td>
</tr>
<tr>
<td>A4 (2x10^9 cfu/g)</td>
<td></td>
<td>12.3</td>
<td>53.8</td>
<td>18.1</td>
<td>18.1</td>
<td>107.3</td>
<td>26.8 (2.7)</td>
</tr>
<tr>
<td>A5 (2x10^10 cfu/g)</td>
<td></td>
<td>11.8</td>
<td>55.6</td>
<td>20.6</td>
<td>20.6</td>
<td>127.7</td>
<td>31.9 (2.8)</td>
</tr>
</tbody>
</table>

* TNF-α levels (pg/mL)

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

<table>
<thead>
<tr>
<th>Factor A (doses)</th>
<th>Factor B (duration of observation)</th>
<th>B1 (0 hour)</th>
<th>B2 (12 hours)</th>
<th>B3 (24 hours)</th>
<th>B4 (36 hours)</th>
<th>Total</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (negative control)</td>
<td></td>
<td>4.0</td>
<td>4.5</td>
<td>4.0</td>
<td>4.0</td>
<td>16.5</td>
<td>4.1 (1.0)</td>
</tr>
<tr>
<td>A2 (positive control)</td>
<td></td>
<td>4.0</td>
<td>6.5</td>
<td>1.5</td>
<td>5.0</td>
<td>17.0</td>
<td>4.3 (1.0)</td>
</tr>
<tr>
<td>A3 (2x10^7 cfu/g)</td>
<td></td>
<td>5.5</td>
<td>6.5</td>
<td>44.5</td>
<td>57.5</td>
<td>114.0</td>
<td>28.5 (2.7)</td>
</tr>
<tr>
<td>A4 (2x10^9 cfu/g)</td>
<td></td>
<td>4.5</td>
<td>5.5</td>
<td>53.5</td>
<td>15.5</td>
<td>79.0</td>
<td>19.8 (2.2)</td>
</tr>
<tr>
<td>A5 (2x10^10 cfu/g)</td>
<td></td>
<td>4.5</td>
<td>4.5</td>
<td>53.0</td>
<td>25.5</td>
<td>87.5</td>
<td>21.9 (2.3)</td>
</tr>
</tbody>
</table>

*LAB (x 10^6 cfu/g)
Aslinar et al: Probiotic W. paramesenteroides on E. coli-induced diarrhea

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 mean E. coli (1.5x10^7 cfu/g) was observed at 36 hours (B4) after administration of 2x10^6 W. paramesenteroides (A3), which was 8-fold lower than the positive control group (A2).

**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. coli serotype O128, reached 30-40 times/day in mice. The study also reported that a probiotic administration could decrease the bowel frequency.\textsuperscript{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 decreased to 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 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.\textsuperscript{17} A study reported that W. paramesenteroides produces bacteriocin, weisellin A, which is active against pathogenic bacteria and protects the intestinal mucosa.\textsuperscript{13}

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

<table>
<thead>
<tr>
<th>Factor A (doses)</th>
<th>Factor B (duration of observation)</th>
<th>Total</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B1 (0 hour)</td>
<td>B2 (12 hours)</td>
<td>B3 (24 hours)</td>
</tr>
<tr>
<td>A1 (negative control)</td>
<td>10.5</td>
<td>12.0</td>
<td>10.5</td>
</tr>
<tr>
<td>A2 (positive control)</td>
<td>11.0</td>
<td>8.0</td>
<td>33.0</td>
</tr>
<tr>
<td>A3 (2x10^6 cfu/g)</td>
<td>8.0</td>
<td>9.0</td>
<td>4.0</td>
</tr>
<tr>
<td>A4 (2x10^6 cfu/g)</td>
<td>6.5</td>
<td>4.5</td>
<td>13.5</td>
</tr>
<tr>
<td>A5 (2x10^10 cfu/g)</td>
<td>7.5</td>
<td>12.0</td>
<td>9.0</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Factor A (doses)</th>
<th>Factor B (duration of observation)</th>
<th>Total</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B1 (0 hour)</td>
<td>B2 (12 hours)</td>
<td>B3 (24 hours)</td>
</tr>
<tr>
<td>A1 (negative control)</td>
<td>5.5</td>
<td>6.0</td>
<td>7.0</td>
</tr>
<tr>
<td>A2 (positive control)</td>
<td>6.0</td>
<td>20.5</td>
<td>19.5</td>
</tr>
<tr>
<td>A3 (2x10^6 cfu/g)</td>
<td>6.5</td>
<td>3.0</td>
<td>2.5</td>
</tr>
<tr>
<td>A4 (2x10^6 cfu/g)</td>
<td>4.5</td>
<td>3.0</td>
<td>3.5</td>
</tr>
<tr>
<td>A5 (2x10^10 cfu/g)</td>
<td>6.0</td>
<td>4.5</td>
<td>4.0</td>
</tr>
</tbody>
</table>

\*Mean E. coli (x10^6 cfu/g)

Enteropathogenic E. coli is the first strain E. coli known to cause diarrhea\textsuperscript{13} when consumed at doses 10^5-10^10 cfu/mL.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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,\textsuperscript{21} or on the seventh day with LAB supplemental, while mice without LAB administration suffered from severe diarrhea.\textsuperscript{22}

Following EPEC administration, we found that TNF-\(\alpha\) levels in mice stool were significantly higher

Paediatr Indones, Vol. 54, No. 1, January 2014 • 5
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. In this study, the mean TNF-α levels were significantly decreased (P<0.01) after W. paramesenteroides administration, at three different doses of 2x10⁸, 2x10⁹, and 2x10¹⁰ cfu/g. After 36 hours study (B4), the group with W. paramesenteroides administration at a dose of 2x10⁸ cfu/g (A3) had the lowest mean level of TNF-α level (15.2 pg/mL), but the groups with higher dose of W. paramesenteroides administration (A4 = 2x10⁹ cfu/g; A5 = 2x10¹⁰ cfu/g) had higher mean levels of TNF-α levels (18.1 pg/mL and 20.6 pg/mL, respectively). These results showed that W. paramesenteroides administration 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. 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. 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, decreased the concentration of TNF-α in the feces,

The mean total LAB levels showed significant differences (P<0.01) between dosing groups for each duration of observation with the highest mean (57.5x10⁷ cfu/g) after 36-hours administration of 2x10⁸ cfu/g W. paramesenteroides, a 11-fold higher than the positive control groups (A2). These findings were consistent with a study reported the highest total LAB in mice with probiotic administration, compared to the group without probiotic administration, before EPEC-induced. Other study also showed an increased amount of LAB in feces after probiotic Lactobacillus plantarum IBI administration. 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.

The mean total aerobic bacteria in our study increased in the positive control group (A2) after 24 hours (B3) EPEC administration to 3.3x10⁹ cfu/g, an increase of 3 times greater than that of the negative control group (10.5x10⁷ cfu/g). Our results were consistent with a study reported dadih, contained 2.8 x 10⁹ cfu/g Lactobacillus, was found to increase the number of colonies of Lactobacillus sp in the duodenum and ileum of mice. 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.

Mean total aerobic bacteria in our study decreased at 36 hours (B4) after administration of 2 x 10⁸ cfu/g W. paramesenteroides to 2.50x10⁷ 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. Other study also found that some isolates of LAB could inhibit pathogenic microorganisms. 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. Another study reported dadih containing LAB was beneficial for killing pathogenic bacteria in the gut. Increased mean total E. coli (up to 20.5x10⁷ 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 10⁷ cfu/g). Mean total E. coli in mice gut was significantly decreased in the groups of mice given 2x10⁸ cfu/g W. paramesenteroides, to 1.50x10⁷ 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
Aslinar et al: Probiotic W. paramesenteroides on E. coli-induced diarrhea

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. 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. 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 2x10⁸ cfu/g. W. paramesenteroides also balances the intestinal microflora in mice with EPEC-induced diarrhea.

Acknowledgements

Our highest gratitude goes to Hendri Purwanto, MS for his assistance with the statistical analysis in this study.

References

22. Astawan M, Bresiyati, Arief I, Sudesti E. Gambaran hematologi tikus putih (Rattus norvegicus) yang diinfeksi
Aslinar et al: Probiotic W. paramesenteroides on E. coli-induced diarrhea


