

Effect of *Lactobacillus casei* subsp. *casei* R-68 Isolated from Dadih on the Procarcinogenic Enzyme Activity and Fecal Microflora Count of Rats Challenged with Pathogenic Bacteria

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Abstract— The human digestive tract is a complex ecosystem that may contain bacteria, yeast, and other microflora, which have harmful and beneficial effects on the host. Species of *Lactobacillus* and *Bifidobacterium* are most commonly used as probiotics. *Lactobacillus casei* subsp. *casei* R-68 (LCR-68) isolated from dadih, traditional fermented buffalo milk from West Sumatera has the potential to be used as probiotic. The purposes of the present study were to evaluate the ability of strain LCR68 to inhibit the growth of the pathogenic bacteria *Listeria monocytogenes* FNCC-0156 and *Escherichia coli* FNCC-19 and reduce the activity of fecal mutagen enzymes in Wistar rats. The *in vivo* test used 25 male Wistar rats with an average weight of 174 - 176 g. This study consisted of five groups of treatment with five rats of each group. The results show a significant increase in the growth in all groups, although a significantly lower weight gain was observed in rats challenged with *Listeria monocytogenes* and fed fermented milk LCR-68. The counts of aerobic and anaerobic microbes were the same in all groups. Significantly higher counts of lactic acid bacteria were determined after the application of fermented milk LCR68. Significantly lower counts of *Escherichia coli* were also observed after the application of fermented milk LCR68. The presence of LCR-68 in fermented milk reduced the activity of β -glucuronidase and β -glucosidase significantly in the feces of Wistar rats. Therefore, the strain R-68 as a probiotic is expected to be able to prevent the formation of procarcinogenic compounds into carcinogens that cause cancer in the digestive tract.

Keywords—dadih; *Lactobacillus casei*; pathogenic bacteria; *in vivo* test; wistar rats.

I. INTRODUCTION

The human digestive tract is a complex ecosystem that may contain bacteria, yeast and other microflora which have harmful and beneficial effects on the host. At present, there are many studies conducted regarding the role of intestinal microflora on the health status of the host [1]. Probiotics are live microorganisms that can provide beneficial effects on the health of their hosts when consumed in sufficient quantities [2] [3] by improving the balance of the intestinal microflora when entering the digestive tract [4] [5]. The mechanism of action of probiotics is by improving the balance of microbes that are already present in the human digestive tract [6]. Species of *Lactobacillus* and *Bifidobacterium* are most commonly used as probiotics [7], but some other bacteria such as *E. coli* [8], some *Bacillus* species [9] [10] [11] and yeast *Saccharomyces species* [12] [13] are also used as probiotics. As a probiotic, *Lactobacillus* has many therapeutic effects, including prevention of cancer through various mechanisms such as binding to mutagens or

carcinogens before these compounds attack normal cells [14] [15] [16], enhancing the immune system through producing immunoglobulin compounds [17] [18] [19] [20] and preventing changes in procarcinogens to carcinogens by inhibiting the growth of enzyme-producing microbes involved in changing these compounds [21] [22] [23]. Generally, microbes involved in the formation of colorectal cancer in the human digestive tract are bacteria which consist of several genera such as Coliforms, *Bacteroides* and *Clostridia* [24]. These bacteria produce enzymes β -glucosidase and β -glucuronidase which convert procarcinogens to carcinogens [25] [26]. Clinical trials show that the administration of *Lactobacillus acidophilus* suppressed the activity of these enzymes, so that it has the potential to prevent colon cancer [27] [28].

Lactobacillus casei subsp. *casei* R-68 (LCR-68) was isolated from dadih, a traditional fermented milk product similar to yoghurt, commonly found in West Sumatera and Kampar Regency of Riau Province, Indonesia [29]. Strain LCR-68 and some dadih's lactic acid bacteria (LAB) have been shown to lower cholesterol through the mechanism of

acid taurocholate deconjugation [30]. Dadih's LAB also has anti-cancer potential because they have antimutagenic properties through binding mechanisms of mutagenic compounds such as N-nitrosodietilamin (NDEA) and N-nitrosopiperidine (NPIP) [14], 3-amino-1,4-dimethyl-5 H-pyrido(4,3-b)indole (Trp-P1) [16], and mutagenic compounds that arise in tauco due to heating at high temperature [15]. These LAB were also resistant to gastric and bile acids or bile acids [15] and could inhibit the growth of *Staphylococcus aureus* FNCC-15, *Listeria monocytogenes* FNCC-0156 and *Escherichia coli* FNCC-19 *in vitro* [31]. The present study reports the ability of LCR68 to inhibit the growth of pathogenic bacteria and the activity of β -glucuronidase and β -glucosidase in rats challenged with *E. coli* and *L. monocytogenes*.

II. MATERIALS AND METHOD

A. Lactic Acid Bacteria and Pathogenic Bacteria

Lactobacillus casei subsp. *casei* R-68 (LCR-68) isolated from dadih by Hosono et al. [29] was used for the present study. The pathogenic bacteria used were *Listeria monocytogenes* FNCC-0156 (Gram-positive bacteria) and *Escherichia coli* FNCC-19 (Gram-negative bacteria). The selection of these two pathogenic bacteria is based on the results of previous *in vitro* studies [31].

B. Activation of LAB and Pathogenic Bacteria Cultures

The active culture was made by aliquoting 0.1 ml of the working culture of LAB into a reaction tube containing 5 ml of MRS Broth, mixing uniformly followed by incubation at 37°C for 18 h. Active culture of LCR68 was used to prepare a starter for the production of probiotic fermented milk. Pathogenic bacteria were activated by inoculating 1 ml of the working culture into 5 ml Nutrient Broth, shaken uniformly and then incubated at 37°C for 18 h.

C. Preparation of Fermented Milk and Skimmed Milk

Probiotic fermented milk was prepared as follows. Skimmed milk (75 g) and CMC (0.05% w/v) was added to water until the volume becomes 500 ml then stirred using a mixer at 1000 rpm for 5 min. The homogenized solution was then sterilized at 105°C for 10 min then cooled to 37°C. Cold skim milk was then inoculated with LCR-68 (5% inoculum) and incubated at 37°C for 15 h to obtain probiotic fermented milk. Skimmed milk was also prepared as described but without inoculation of LCR-68.

D. Animal Test

An *in-vivo* study for antimicrobial activity of LCR-68 was conducted according to Sreekumar and Hosono [23]. The *in vivo* test used 25 male Wistar Rats weighing 120-150 g. The pathogenic bacteria selected in the *in vivo* test were *Listeria monocytogenes* FNCC-0156 and *Escherichia coli* FNCC-19 that were previously shown to be sensitive to antimicrobial compounds produced by LCR-68 in *in vitro* study [31]. All rats were given a basal chow diet for 5 days before being divided into 5 groups of 5 rats each. Group 1 (control group) was given a commercial diet and skimmed milk (without LCR-68), Group 2 was treated with commercial diet, skimmed milk and cell suspension of *E. coli* FNCC-19

(about 2×10^5 cfu/ml), Group 3 was treated with commercial diet, skimmed milk and cell suspension of *L. monocytogenes* FNCC-0156 (about 2×10^5 cfu/ml), Group 4 was treated with commercial diet, fermented milk LCR-68 (containing about 5×10^7 cfu/ml) and cell suspension of *E. coli* FNCC-19 (about 2×10^5 cfu/ml) and Group 5 were treated with commercial diet, fermented milk LCR-68 (containing about 5×10^7 cfu/ml) and cell suspension of *L. monocytogenes* FNCC-0156 (about 5×10^5 cfu/ml). In each treatment, rats were fed skimmed milk or fermented milk LCR-68 for 5 days, then given pathogenic bacteria according to treatments for 4 days. After that, the rats were only given a commercial diet for 2 days in a row. Skimmed milk, fermented milk LCR-68 and bacterial pathogen suspension were administered to rats by the oral gavage method of 0.5 ml/day. Fecal samples were collected before, during and after treatment. The weight of each rat was weighed just before dividing into groups and at the end of the study. Animal care was in accordance with the guidelines for Animal Experimentation of the Faculty of Medicine, University of Riau, Pekanbaru Indonesia.

E. Analysis of Microbes in Feces

Fresh fecal samples were taken from each rat by gently pressing the rectal part of the rat rectum. The fecal sample was put into a test tube and then closed tightly and analyzed within 30-60 min. The samples were homogenized and diluted using a sterile phosphate buffer. MRS Agar was used for enumeration of LAB, Eosin Methylene Blue (EMB) Agar for *E. coli*, Listeria Selective Palcam Agar for *L. monocytogenes* and PCA medium for total aerobic and anaerobic microbes. All plates were incubated at 37°C for 2 days. The same method and media was used for enumeration of both aerobic and anaerobic bacteria with slight modification. The counts of anaerobic microbes was enumerated by adding a layer of sterile agar of about 40-45°C above the agar plates that have been inoculated to create an aerobic conditions inside the agar plates. After that, the mediums were allowed to solidify and then incubated at 37°C for 2 days.

F. Analysis of Enzymes in Feces

Preparation of fecal samples for enzyme analysis were similar to those for microbial analysis purposes. Fresh fecal samples were then stored in the refrigerator for 1 day. The fecal samples were weighed and 0.5 g added to 3 ml of a 0.1 M potassium phosphate buffer (pH 7.0) and homogenized for 15 min and then centrifuged at 5,000 rpm for 15 min to obtain fecal supernatant. Fecal supernatants were analyzed for β -glucuronidase and β -glucosidase enzyme activity according to the method described by Sreekumar and Hosono [23]. Both enzymes play an important role in converting procarcinogen compounds into cancer-causing carcinogens, especially colon cancer.

G. Assay for β -glucuronidase Activity

A total of 0.1 ml fecal supernatants were mixed with 0.02 M potassium phosphate buffer, 0.1 mM EDTA, 1 mM phenolphthalein- β -D-glucuronide to obtain a 1 ml reaction mixture. The reaction was allowed to proceed for 30 min at 37°C. The reaction was stopped by adding 5 ml of 0.2 M

glycine buffer (pH 10.4) containing 0.2 M NaCl. The absorbance was measured using a spectrophotometer at 540 nm. The amount of phenolphthalein released was calculated by comparing the standard curve of phenolphthalein. The specific activity of this enzyme is expressed in $\mu\text{mol}/\text{mg}$ of protein per 30 min.

H. Assay for β -glucosidase Activity

A total of 0.2 ml fecal supernatants were mixed with potassium phosphate buffer 0.1 M and nitrophenyl- β -D-glucoside 1 mM to obtain a 1 ml reaction mixture. The reaction was allowed at 37°C for 30 min. The reaction was stopped by adding 5 ml of 0.01 M NaOH. The absorbance was measured using a spectrophotometer at 420 nm. The amount of nitrophenol produced was calculated by comparing the standard curve of nitrophenol. The specific activity of this enzyme is expressed in $\mu\text{mol}/\text{mg}$ of protein per 30 min.

I. Statistical Analyses

The data were analyzed by the ANOVA procedure from SPSS version 16. The least significant difference procedure was used to determine if statistically significant differences occurred among means at a level of $P < 0.05$.

III. RESULTS AND DISCUSSION

Fig. 1 show the effect of skimmed milk and fermented milk LCR-68 in rats challenged with pathogens on body weight and weight gain of rat. During the experimental period, no clinical signs of disorder or disease were observed in any of the groups.

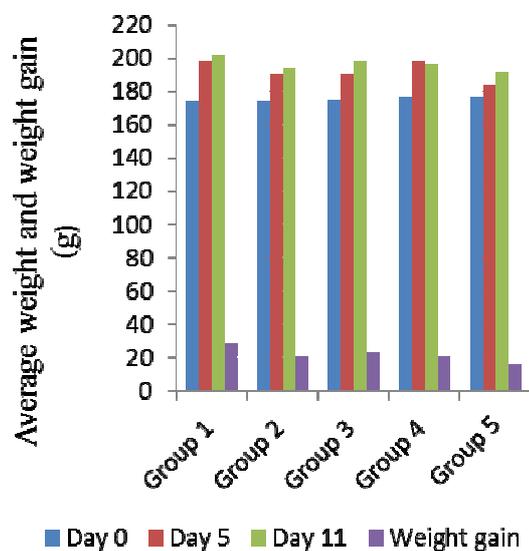


Fig 1. Weight gain of rat challenged with *Escherichia coli* FNCC-19 and *Listeria monocytogenes* FNCC-0156 with or without feeding fermented milk LCR-68

The data in Figure 1 show show significant growth ($P < 0.05$) starting from day 5 to 11, growth on day 5 and day 11 was not significantly different ($P < 0.05$). In addition, the weight of rats for all groups showed no significant difference ($P < 0.05$) between each other on day 11. Although growth showed no significant difference ($P < 0.05$), weight gain showed a significant decrease ($P < 0.05$) in group 5 compared

to group 1 (control). This may because the rats in the control group did not experience gastrointestinal disorders due to the presence of pathogenic bacteria. The nutrient compounds especially lactose and proteins in the skimmed milk remained intact and this is thought to have been a major factor in the increased weight gain of rats in group 1. The same result also was obtained in rats fed skimmed milk [30]. The lowest weight gain occurred in group 5, but was not significantly different with groups 2, 3 and 4. This may be due to the presence of *E. coli* and *L. monocytogenes* which disturbed the balance of microflora in the rat intestine, which in turn affects the digestion process of food and absorption of nutrients. Another possibility is that these pathogenic bacteria use some nutrients in non skimmed milk and fermented milk for their growth. This result is somewhat contradictory to Oyetayo [32] research results that reported weight gain in rats challenged with *E. coli* and along with several strains of *L. acidophilus* isolated from pigs, albino rats and neonatal infants. The decrease in weight gain in a group containing diet containing LAB was also reported by Pato and Hosono [30] in rats fed fermented milk made from *Lactococcus lactis* subsp. *lactis* IS 10285, Xie et al. [33] in rats given high-cholesterol diet + *Lactobacillus plantarum* 9-41-A; in rats given high-cholesterol diet + *B. longum* SPM1207 [34]. In contrast Konstantinov et al. [35] reported increased growth in the piglet ileum fed a diet containing *Lactobacillus sobrius* DSM 16698; Salaj et al. [36] reported no effect of *L. plantarum* LS/07 and *Lactobacillus plantarum* Biocenol LP96 on the growth and weight gain in SD rats.

Faecal samples of the rats were collected to compare the counts of certain microbes. The effect of skimmed milk and fermented milk LCR-68 in rats challenged with pathogens on counts of LAB in feces of rats is presented Table 1.

TABLE I
COUNTS OF LACTIC ACID BACTERIA IN RATS CHALLENGED WITH *ESCHERICHIA COLI* FNCC-19 AND *LISTERIA MONOCYTOGENES* FNCC-0156 WITH OR WITHOUT FEEDING FERMENTED MILK LCR-68

Groups	Average counts of lactic acid bacteria (log cfu/gr)		
	Initial treatment (day 0)	During treatment (day 5)	End treatment (day 11)
Group 1	^a 7.01±0.47 ^A	^b 7.63±0.06 ^A	^a 7.31±0.34 ^A
Group 2	^a 7.34±0.45 ^A	^{ab} 7.49±0.49 ^A	^a 7.13±0.36 ^A
Group 3	^a 6.82±0.59 ^A	^a 7.02±0.40 ^A	^a 7.14±0.32 ^A
Group 4	^a 6.60±0.19 ^A	^b 7.72±0.39 ^B	^b 7.79±0.24 ^C
Group 5	^a 7.02±0.27 ^A	^{ab} 7.51±0.32 ^B	^b 7.74±0.25 ^B

Means followed by the lowercase letters in the same column and uppercase letters in the same line indicated significant difference ($p < 0.05$).

The counts of LAB in groups 1, 2 and 3 significantly increase ($P < 0.05$) from day 5 to 11. This is due to the absence of dietary intake containing strain LCR-68 in these 3 groups. In contrast to groups 4 and 5, there was significant increase ($P < 0.05$) in the counts of LAB starting from d 5 to 11, originating from the intake of fermented milk containing strain LCR-68. It is suspected that LCR-68 was able to grow well in the digestive tract of rats because this strain was resistant to acid and bile [30]. Similar study have also

reported an increase in the counts of LAB in rat stools [34], *Lactobacilli* and *Bifidobacterium* in the intestinal tract of rats [33] [37]; *Lactobacilli* in rat feces [38].

The effect of skimmed milk and fermented milk LCR-68 in rats challenged with pathogens on counts of aerobic and anaerobic microbe (Table 2 and 3) in feces of rats.

TABLE II
COUNTS OF AEROBIC MICROBES IN RATS CHALLENGED WITH *ESCHERICHIA COLI* FNCC-19 AND *LISTERIA MONOCYTOGENES* FNCC-0156 WITH OR WITHOUT FEEDING FERMENTED MILK LCR-68

Groups	Average counts of aerobic microbes (log cfu/gr)		
	Initial treatment (day 0)	During treatment (day 5)	End treatment (day 11)
Group 1	^a 6.75±0.87 ^A	^b 7.41±0.29 ^A	^a 7.00±0.31 ^A
Group 2	^a 6.77±0.42 ^A	^a 6.77±0.22 ^A	^a 7.81±0.32 ^B
Group 3	^a 6.38±0.39 ^A	^{ab} 6.95±0.41 ^{AB}	^a 7.18±0.62 ^B
Group 4	^a 6.80±0.63 ^A	^a 6.83±0.21 ^A	^a 7.22±0.63 ^A
Group 5	^a 6.81±0.53 ^A	^b 7.30±0.45 ^A	^a 7.00±0.86 ^A

Means followed by the lowercase letters in the same column and uppercase letters in the same line indicated a significant difference (p<0.05).

Table 2 shows that the counts of aerobic microbes in all treatments did not increase significantly (P<0.05) during treatment from day 5 to 11, except in Groups 2 and 3 that significantly increased (P<0.05) on day 11. The Increase in the counts of aerobic microbes in groups 2 and 3 may be due to *E. coli* and *L. monocytogenes* being able to survive and grow in the digestive tract of rats. However, the increase in the counts of aerobic microbes in groups 2 and 3 were not significantly different (P<0.05) when compared with the total counts of aerobic microbes in groups 1, 4 and 5 on day 11. This is maybe due to the increase in the counts of certain microbes in all groups that may contribute to the increase in the counts of aerobic microbes. The increase in the number of aerobic microbes in group 1 was mostly from normal microflora living in the gastrointestinal tract of the rat, which is facultative aerobic bacteria such as *Coliform*, *Enterococcus*, *Pseudomonas*, *Proteus*, *Lactobacilli*, and also yeasts such as *Candida* and other microorganisms. The increase in the counts of aerobic microbes in Group 2 and 3 may be due to the increase in the counts of *E. coli* and *L. monocytogenes*, and in Groups 4 and 5 may be due to the increase in the counts of BAL especially *L. casei* subsp. *casei* R-68, as shown in Table 3. Thus the total counts of aerobic microbes in all groups were similar on day 11. Similar results were reported by Haberer et al. [39] in minipig stools fed high-cholesterol diet followed by the diet containing a mixture of three *Lactobacillus* strains.

Counts of anaerobic microbes in groups 1, 2, 3, and 5 on day 11 did not increase significantly (P<0.05) on d 5 after skimmed milk or fermented milk LCR-68 intake after being challenged by pathogenic bacteria (Table 3). This indicates that rats challenged by pathogens with or without strain LCR-68 did not affect the number of anaerobic microbes such as *Bacteroides*, *Fusobacteria*, *Enterobacteriaceae* and other anaerobic bacteria in the intestinal tract. However, the counts of anaerobic microbes increased significantly (P<0.05) in Group 4 on day 11 fed *E. coli* as well as fermented milk

LCR-68. This is likely due to the presence of strain LCR-68 which is known to be capable of suppressing *E. coli* growth and simultaneously stimulating the growth of anaerobic microbes in the intestinal tract so that the total amount of aerobic microbes increased significantly (P<0.05) at the end of treatment (day 11).

TABLE III
COUNTS OF ANAEROBIC MICROBES IN RATS CHALLENGED WITH *ESCHERICHIA COLI* FNCC-19 AND *LISTERIA MONOCYTOGENES* FNCC-0156 WITH OR WITHOUT FEEDING FERMENTED MILK LCR-68

Groups	Average counts of anaerobic microbes (log cfu/gr)		
	Initial treatment (day 0)	During treatment (day 5)	End treatment (day 11)
Group 1	^a 7.30±0.62 ^A	^a 7.42±0.12 ^A	^a 7.53±0.10 ^A
Group 2	^a 7.27±0.75 ^A	^a 7.37±0.23 ^A	^a 7.47±0.41 ^A
Group 3	^a 7.45±0.34 ^A	^a 7.29±0.12 ^A	^a 7.77±0.58 ^A
Group 4	^a 6.98±0.67 ^A	^a 7.64±0.48 ^{AB}	^a 7.81±0.23 ^B
Group 5	^a 7.02±0.61 ^A	^a 7.37±0.88 ^A	^a 7.29±0.42 ^A

Means followed by the lowercase letters in the same column and uppercase letters in the same line indicated a significant difference (p<0.05).

This statement is supported by the present research data in Tables 4 and 5, which show a significant decrease (P<0.05) in the counts of *E. coli* and *L. monocytogenes* in groups 4 and 5. The increase in the counts of anaerobic microbes was also observed in the minipig feces given a high-cholesterol diet followed by a diet containing a mixture of three *Lactobacillus* strains [39].

The effect of skimmed milk and fermented milk LCR-68 in rats challenged with pathogens on counts of *E. coli* in the feces of Wistar rats is presented in Table 4.

TABLE IV
COUNTS OF *E. COLI* IN RATS CHALLENGED WITH *ESCHERICHIA COLI* FNCC-19 AND *LISTERIA MONOCYTOGENES* FNCC-0156 WITH OR WITHOUT FEEDING FERMENTED MILK LCR-68

Groups	Average counts of <i>E. coli</i> (log cfu/gr)		
	Initial treatment (day 0)	During treatment (day 5)	End treatment (day 11)
Group 1	^a 6.71±0.67 ^A	^a 6.54±0.56 ^A	^b 6.49±0.64 ^A
Group 2	^a 6.90±0.60 ^A	^a 6.59±0.59 ^A	^b 6.44±0.50 ^A
Group 3	^a 6.76±0.74 ^A	^a 6.29±0.70 ^A	^b 6.82±0.31 ^A
Group 4	^a 6.68±0.73 ^A	^a 6.72±0.62 ^A	^{ab} 6.31±0.61 ^A
Group 5	^a 7.19±0.65 ^B	^a 6.28±0.39 ^A	^a 6.11±0.09 ^A

Means followed by the lowercase letters in the same column and uppercase letters in the same line indicated a significant difference (p<0.05).

The counts of *E. coli* in groups 1, 2, and 3 did not change significantly (P<0.05) from d 0 to 11, whereas in groups 4 and 5, there was a significant decrease (P<0.05) in *E. coli* on day 11. This is due to the strain LCR-68 in the dietary intake of rats capable of inhibiting the growth of *E. coli* in the intestinal tract of rats so that the counts of *E. coli* decreased significantly (P<0.05). This is in line with a previous *in vivo* study that demonstrated the ability of strain LCR-68 to inhibit *E. coli* growth [31]. The results of this study are consistent with the results reported by Sreekumar and Hosono [23] who reported a decrease in the counts of *E. coli*

in rats fed *L. acidophilus* SBT2074 challenged with *E. coli*; Xie et al. [33] in rats fed high-cholesterol diet + *Lactobacillus Plantarum* 9-41-A; Bian et al. [37] in *E. coli* O157: H7-infected rats + *Lactobacillus acidophilus* and *Lactobacillus helveticus*; Konstantinov et al. [35] in the piglet ileum fed with *Lactobacillus sobrius* DSM 16698.

The effect of skimmed milk and fermented milk LCR-68 in rats challenged with pathogens on counts of *Listeria monocytogenes* in the feces of Wistar rats is presented in Table 5.

TABLE V
COUNTS OF *LISTERIA MONOCYTOGENES* IN RATS CHALLENGED WITH *ESCHERICHIA COLI* FNCC-19 AND *LISTERIA MONOCYTOGENES* FNCC-0156 WITH OR WITHOUT FEEDING FERMENTED MILK LCR-68

Groups	Average counts of <i>Listeria monocytogenes</i> (log cfu/gr)		
	Initial treatment (day 0)	During treatment (day 5)	End treatment (day 11)
Group 1	^a 6.63±0.38 ^B	^a 6.02±0.34 ^A	^b 6.74±0.25 ^B
Group 2	^a 6.60±0.23 ^B	^a 5.82±0.28 ^A	^{ab} 6.53±0.42 ^B
Group 3	^a 6.53±0.68 ^A	^a 5.94±0.27 ^A	^{ab} 6.55±0.40 ^A
Group 4	^a 6.76±0.55 ^B	^a 6.06±0.18 ^A	^a 6.12±0.39 ^A
Group 5	^a 6.83±0.45 ^B	^a 6.01±0.36 ^A	^a 6.10±0.50 ^A

Means followed by the lowercase letters in the same column and uppercase letters in the same line indicated significant difference (p<0.05).

The counts of *L. monocytogenes* in groups 1, 2, and 3 did not change significantly (P<0.05) between day 0 to day 11, although the counts of *L. monocytogenes* in groups 1 and 2 decreased significantly (P<0.05) on day 5. A significant decrease (P<0.05) in the counts of *L. monocytogenes* occurred in Groups 4 and 5 fed fermented milk LCR-68. This suggests that strain LCR-68 had the ability to inhibit the growth of *L. monocytogenes* in this *in vivo* study. The findings of the present study were in accordance with the resulting study by Waard et al. [40] who reported that *Lactobacillus casei* Shirota strain YIT9029 reduced the counts of *L. monocytogenes* not only in the feces but also in the stomach, caecum, spleen, and liver of rats.

TABLE VI
B-GLUCURONIDASE ACTIVITY IN RATS CHALLENGED WITH *ESCHERICHIA COLI* FNCC-19 AND *LISTERIA MONOCYTOGENES* FNCC-0156 WITH OR WITHOUT FEEDING FERMENTED MILK LCR-68

Groups	β-glucuronidase activity (μmol/mg dari protein per 30 menit)		
	Initial treatment (day 0)	During treatment (day 5)	End treatment (day 11)
Group 1	^a 0.954±0.166 ^A	^a 0.802±0.114 ^A	^a 0.937±0.1768 ^A
Group 2	^a 0.859±0.153 ^A	^b 1.102±0.126 ^B	^b 1.049±0.184 ^{AB}
Group 3	^a 0.937±0.088 ^A	^b 0.986±0.082 ^A	^b 1.032±0.100 ^A
Group 4	^a 0.987±0.202 ^A	^a 0.818±0.210 ^A	^a 0.879±0.113 ^A
Group 5	^a 0.900±0.093 ^A	^a 0.838±0.093 ^A	^a 0.825±0.085 ^A

Means followed by the lowercase letters in the same column and uppercase letters in the same line indicated significant difference (p<0.05).

Cancer is one of the biggest causes of death in humans. Colon cancer is one type of cancer caused by carcinogenic compounds in the colon. Some microbes, especially

pathogenic bacteria were capable of converting pro-carcinogen compounds into cancer-causing carcinogens involving several enzymes such as β-glucuronidase and β-glucosidase. Rats challenged with pathogens fed on fermented milk LCR-68 showed a significant (P<0.05) increase in the β-glucuronidase activity in the feces of Wistar rats (Table 6).

β-Glucuronidase activity in groups 1 and 3 tended to increase on day 11, but the increase was not significant (P<0.05). In group 2, there was a significant increase (P<0.05) in β-glucuronidase activity on d 5, but activity tended to decreased again on day 11. The increased activity in β-glucuronidase was due to the production of this enzyme by rat intestinal bacteria and pathogenic bacteria given to rats. The major producers of β-glucuronidase are intestinal bacteria, especially *Escherichia coli*, *Clostridium paraputrificum*, *Clostridium clostridioforme*, *Clostridium perfringens*, *Bacteroides fragilis*, *Bacteroides vulgatus*, *Bacteroides uniformis*, *Ruminococcus gnavus*, *Peptostreptococcus*, *Staphylococcus* and *Eubacterium* [41] [42]; *Lactobacillus* spp and *Enterococcus* spp [22]. β-glucuronidase was produced by several unidentified Gram-negative bacteria, *Bacillus* species, *Escherichia coli*, Proteobacteria, and Enterobacteriaceae species [43] [44]. In groups 4 and 5, the β-glucuronidase activity tended to decrease from day 0 to 11 even though the decrease was not significant (P<0.05). On day 11, rats in groups 4 and 5 challenged with pathogenic bacteria and fed fermented milk LCR-68 had significantly lower (P<0.05) β-glucuronidase activity compared to groups 2 and 3, groups challenged with the pathogenic bacteria *E. coli* and *L. monocytogenes* without feeding fermented milk LCR-68. The decrease in β-glucuronidase activity was due to the decrease in the counts of enzyme-producing bacteria, especially pathogenic bacteria like *E. coli* and *L. monocytogenes* as shown in Table 3. Sreekumar and Hosono [23] reported a decrease in β-glucuronidase activity observed in the small intestine and caecum in rats challenged with *E. coli* and fed *L. acidophilus* SBT2074. Decreased activity of β-glucuronidase had also been reported in rats fed a high-fat diet containing carcinogen and *L. acidophilus* KFRI342 [45]; in fresh caecal digesta of male SD rats fed combination of antibiotic and probiotic *Lactobacillus plantarum* LS/07 [46]; in caecum of rats supplemented with *L. acidophilus* NCFM [47]; in rat feces given only *L. plantarum*, *L. plantarum* + inulin or *L. plantarum* + line oleum virginale [38]; in rat stools given high-cholesterol diet + *B. longum* SPM1207 [32]; in rat caecum fed *Lactobacillus* GG + DMH and *L. acidophilus* + DMH-treated rats [48]. While Salaj et al. [36] reported no effect of *L. plantarum* LS/07 and *L. plantarum* Biocenol LP96 on β-glucuronidase activity in high fat diet-treated rats.

β-glucosidase activity increased significantly (P<0.05) from day 0 to 11 in all groups, but the increase in β-glucosidase activity in groups 4 and 5 were significantly smaller (P<0.05) than that in groups 1, 2 and 3 (Table 7).

TABLE VII
B-GLUCOSIDASE ACTIVITY IN RATS CHALLENGED WITH *ESCHERICHIA COLI* FNCC-19 AND *LISTERIA MONOCYTOGENES*

Groups	β-glucosidase activity (μmol/mg dari protein per 30 menit)		
	Initial treatment (day 0)	During treatment (day 5)	End treatment (day 11)
Group 1	^a 1.304±0.359 ^A	^b 2.947±0.301 ^B	^b 3.69±1.099 ^B
Group 2	^a 1.408±0.256 ^A	^b 2.969±0.652 ^B	^b 2.89±1.251 ^B
Group 3	^a 1.424±0.197 ^A	^{ab} 2.847±0.235 ^B	^b 3.469±0.885 ^B
Group 4	^a 1.578±0.198 ^A	^{ab} 2.773±0.339 ^B	^{ab} 2.59±0.890 ^B
Group 5	^a 1.548±0.293 ^A	^a 2.297±0.412 ^B	^a 2.003±0.556 ^{AB}

Means followed by the lowercase letters in the same column and uppercase letters in the same line indicated a significant difference (p<0.05).

The increased activity in β-glucosidase may be due to the production of this enzyme by pathogenic bacteria administered to rats and or by intestinal bacteria mainly from genera *Clostridium spp.* and *Bacteroides spp.* *Enterococcus spp.*, *Bifidobacterium spp.*, *Lactobacillus spp.*, such as *Bacteroides uniformis*, *Bacteroides ovatus*, *Clostridium paraputrificum*, *Clostridium clostridioformes*, *Enterococcus faecalis* [42] [22] [49]. Thus on d 11, groups 4 and 5 had significantly lower (P<0.05) β-glucosidase activity than groups 1, 2 and 3. These results indicate that strain LCR-68 contained in fermented milk was capable of decreasing the activity of β-glucosidase through inhibiting the enzyme producers, namely *E. coli* and *L. monocytogenes* as shown in Table 3. A decrease in β-glucosidase activity also occurred in the caecum of rats challenged with *E. coli* and fed *L. acidophilus* SBT2074 [23]; in the stools of rats received high-fat diet containing carcinogen and *L. acidophilus* KFRI342 [44]; in the caecum of rats supplemented with *L. acidophilus* NCFM [47]; in rat feces fed only *L. plantarum*, *L. plantarum* + inulin or *L. plantarum* + line oleum virginale [38]; in rat stools fed high-cholesterol diet + *B. longum* SPM1207 [21]. However, contradictory results by Hijova et al. [46] were published, the authors reported an increase in β-glucosidase activity in fresh caecal digesta of male SD rats given a combination of antibiotic and probiotic *Lactobacillus plantarum* LS/07.

Bacterial β-glucosidases seem to be more widespread among the gut microflora than β-glucuronidases [21]. This statement is consistent with the present finding which the activity of β-glucuronidases was higher β-glucosidases (shown in Tables 6 and 7). The change in the number of intestinal microbiota (shown in Tables 1, 2, 3, 4 and 5) may induce an increase or a decrease of the activity level of β-glucuronidases, β-glucosidases and possibly other enzymes, and thus they influence the presence of toxic and often carcinogenic substances in an organism.

IV. CONCLUSIONS

A significant increase in the growth of rats in all groups occurred although growth was significantly lower in rats challenged with *L. monocytogenes* and fed fermented milk LCR-68. LCR-68 maintained the counts of aerobic and anaerobic microbes, increased significantly the counts of lactic acid bacteria and decreased significantly the counts of *E. coli* and *L. monocytogenes* as well as a significant reduction in the activity of β-glucuronidase and β-

glucosidase enzymes in rats challenged with *E. coli* and *L. monocytogenes*.

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