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Efficacy of Encapsulated *Lactobacillus casei* Probiotics as Anti Diarrheal Agent on Sprague Dawley Rats

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Abstract: The aim of this study was to evaluate the effectiveness of encapsulated Lactobacillus casei probiotics isolated from dadih (West Sumatra traditional yoghurt) against diarrhea caused by Escherichia coli. Total 30 male Sprague-dawley rats were divided into five groups (1) normal control rats, (2) infected rats, (3) normal rats with encapsulated probiotics, (4) infected rats with encapsulated probiotics, (5) infected rats with encapsulated probiotics given simultaneously. Rats were fed with standard diet and aquades *ad libitum*. Infected rats were challenged by *E. coli* (10^8 cfu/ml) for 7 days daily orally and then administered by 10^9 cfu/g probiotics for the next 7 days. On day 0, 3 and 7, total of fecal lactic acid bacteria (LAB) and *E. coli* were evaluated. Feed intake, weight gain and food conversion efficiency (FCE) were also evaluated. At the end of treatment rats were sacrificed to observed goblet cells count obtained from ileum. It was observed that encapsulated Lactobacillus casei could increase FCE and total LAB. Encapsulated Lactobacillus casei also could reduce *E. coli* population and reduce total goblet cells on infected rats. Encapsulated Lactobacillus casei has potential effect as probiotics against *E. coli* on rats even it is not statistically different.

Key words: Encapsulated probiotic, *Lactobacillus casei*, diarrhea, sprague dawley

INTRODUCTION

Diarrhea is globally known as the cause of morbidity and mortality among children under five years of age (Black *et al*., 2010). Fischer Walker *et al*. (2012) mentioned that globally, diarrhea incidence among under five years old children in 2010 reached 1.7 million. In Africa, the diarrheal infection was 7 times per year, higher than other developing countries with frequency of 3 times per year (Casburn-Jones and Farthing, 2004). Ahmed *et al*. (2008) mentioned that prevalence of diarrhea among children under five years old in Kashmir India was 25.2%. According to Riskesdas 2007, the biggest cause of mortality among children under five years old in Indonesia was diarrhea with prevalence of 25.2%. Lanata *et al*. (2013) mentioned that more than half of deaths among children underfive in the world due to diarrhea were caused by viral infection (rotavirus and calicivirus) and bacterial infection, particularly *Eschericia coli*.

Diarrhea and malnutrition is strongly related. Improper management of acute diarrhea among children will cause chronic diarrhea and damage in nutrient absorption leading to child malnutrition. On the contrary, in malnourished children, the immunity is impaired causing susceptibility to diarrhea. As the result, vicious cycle of diarrhea-malnutrition-diarrhea occurs. When the condition is not improved, it will lead to child mortality (Rieuwpassa, 2005).

Probiotics are known effective to control microorganism growth potential as pathogen which cause diarrhea. Probiotics were able to control various enteric pathogens, such as *Salmonella typhimurium*, *Shigella*, *Clostridium difficile*, *Campylobacter jejuni* and *Escherichia coli* (Bengmark, 1998). Moreover, some studies also showed potential of isolated lactic acid bacteria to reduce diarrhea caused by pathogen infection, viral infection and antibiotics intake (Heyman and Menard, 2002).

One of the ways to maintain number of probiotics during product development and storage is by doing encapsulation. Probiotic encapsulation is process of creating capsule from active material in the form of solid, liquid or dispersion covered by thin layer functioned to prevent damage caused by microbe due to the environment. Cell encapsulation process can reduce vitality and stability of microbes during production, management and storage processes (Kailasapathy, 2002). McFarland and Elmer (2006) stated that based on clinical evidence, in order to give effect on health, the recommended dosage of probiotics was 10^8-10^{10} cfu/day. Effect of encapsulated probiotics on health was necessary to be assessed. Therefore, this study conducted *in vivo* test to know the effect of encapsulated probiotics feeding on *Feed Conversion Efficiency* (FCE), total fecal lactic acid bacteria, total fecal *E. coli* and total goblet cell on rats.

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MATERIALS AND METHODS

Time and place: This study was part of big study entitled Functional Food Development: Blondo Based Probiotic Biscuit to Improve Nutritional Status and Immunity among Children Under Five Years in South Sulawesi Province. The study was conducted in September-November 2014 in Laboratory of Microbiology in Indonesian Center for Agricultural Postharvest Research and Development, Laboratory of Histopathology in Faculty of Veterinary of Bogor Agricultural University and Food Quality and Safety Laboratories in SEAFAST Center of Bogor Agricultural University.

Materials and tools: Materials used in this study included *Lactobacillus casei* isolated from dadih (West Sumatra traditional yoghurt) came from Sijunjung District in West Sumatra Province, *Escherichia coli* culture from calves in Faculty of Veterinary Laboratory of Bogor Agricultural University, sodium alginate, powdered skim milk and $CaCl_2$. Animal used for experimentation was *Sprague Dawley* rats. The tools for analysis were incubator, autoclave, *laminar flow*, vortex, pH meter and oven. The tools for *in vivo* test rat cage, water bottle, food bowl and feeding tube to insert *E. coli*.

Procedure: The study was started by creating encapsulated probiotics. Probiotics used in this study was *Lactobacillus casei* isolated from dadih (West Sumatra traditional yoghurt) came from Sijunjung District in West Sumatra Province. Encapsulation process was conducted using extrusion technique with encapsulation material of sodium alginate-skim milk suspension. Drying was conducted using oven with temperature of 40°C to obtain powdered encapsulated probiotics.

In vivo test was conducted to analyze effectiveness of the encapsulated probiotics. Thirty male *Sprague Dawley* rats aged 35-42 days were used in this study. All rats were given adaptation process for five days. Each rat was placed individually in the cage with one water bottle and food bowl per cage. The rats were given food and drink *ad libitum*. Standard diet based on Harlan (2008) was given. The average weight of rats was 64.9±2.9 gram. All rats were randomly assigned to five groups with specific treatments (Table 1). The rats were weighed in every two days. The leftovers was collected and weighed to calculate total consumption.

Infection of *E. coli* was given to rats in group B and D during 7 consecutive days with dosage of 10^8 cfu/ml. Encapsulated probiotics was given to rats in group C and D once a day during the next consecutive days. In group E, the exposure of *E. coli* and probiotics was given simultaneously. Encapsulated probiotics with dosage of 10^9 cfu/g was given in the form of powder added to 1 g of food to ensure that the food is totally consumed by the rats. Stoll test was conducted on day 0, 3 and 7 by analyzing total lactic acid and coliform bacteria. Ileum histopathology was conducted by rat necropsy on the last experiment day. The ileum of rats were taken and made into histopathology slides. The slide preparation included fixing, processing, embedding, sectioning and staining. The slides were observed with 200 times magnification using microscope, captured and total goblet cell was calculated using Image J software. The study was approved by Animal Ethics Committee of Bogor Agricultural University with Number 7-2014.

Table 1: Grouping and treatment of rats

Group	Treatment	No. of rats
A	Without *E. coli*, without probiotics	6
B	With *E. coli*, without probiotics	6
C	Without *E. coli*, with probiotics	6
D	With *E. coli*, with probiotics	6
E	With *E. coli* and probiotics simultaneously	6

Table 2: Food intake, weight gain and FCE value of rats during treatment

Group	Food intake (g)	Weight gain (g)	FCE (%) (WeightGain/food intake x 100%)
A	92.2±10.2	34.0±4.3	37.0±4.2
B	90.0±11.3	32.8±4.4	36.5±2.7
C	74.2±12.7	31.3±7.9	41.7±5.1
D	87.6±12.7	34.5±6.9	39.3±5.7
E	81.9±7.9	29.4±4.7	35.7±3.7

Analysis: Data whose design completely randomized was analyzed using Microsoft Excel 2007 and SAS 9.31 for Windows. Data was presented in mean±standard deviation. Statistical test used in this study was Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Feed conversion efficiency (FCE): FCE (Feed Conversion Efficiency) is the ratio between weight gain and food intake in each groups. Higher the FCE value, more efficient is the diet to increase weight gain of rats. Food intake, weight gain and FCE value of rats during the treatment was presented in Table 2.

Encapsulated *Lactobacillus casei* probiotics had potential to improve FCE value of the rats even though it was not significantly different ($p>0.05$). Table 2 showed that FCE value of rats with probiotics was higher than those who did not given probiotics. FCE value of healthy rats given probiotics (C) was higher than the rats with standard diet (A). Similarly, the value was higher in rats given probiotics (D) than the standard diet (B). The FCE value in Group E showed the lowest FCE value among all groups. This may be due to the exposure of both *E. coli* and probiotics in the same time so that the activity of probiotics was impaired.

Probiotics from *Lactobacillus* genus is predicted to be able to improve consumption of rats due to some factors, such as by being able to improve nutrient absorption by producing digestion enzymes, especially proteolytic enzymes. Parvez *et al*. (2006) mentioned that

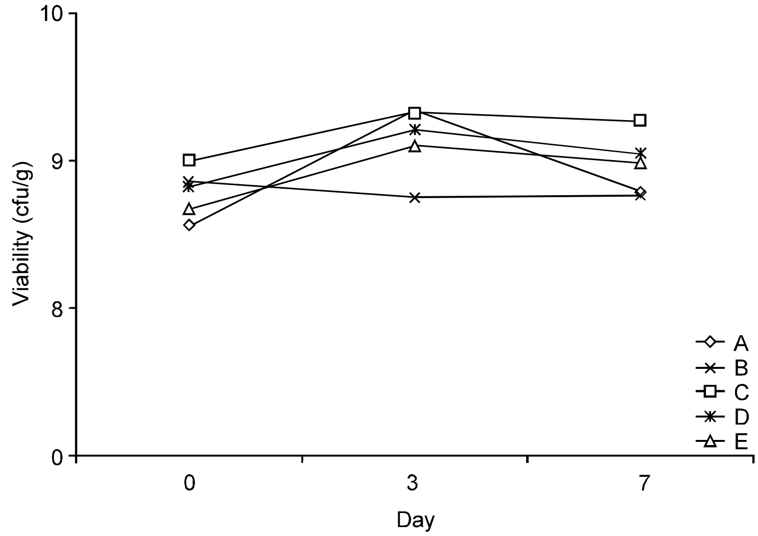

Fig. 1: Total lactic acid bacteria (LAB) during treatment. ◆: without *E. coli*/without probiotics, x: with *E. coli*/without probiotics, ■: without *E. coli*/with probiotics, *: with *E. coli*/with probiotics, ▲: with *E. coli* and probiotics simultaneously

probiotics can give advantage to the host by synthesizing vitamin and releasing amino acid to support growth of the host. This improved weight gain of the rats. Gross *et al*. (2008) using *Lactobacillus plantarum* 299v probiotics showed that probiotics improved weight gain and food intake of rats. Moreover, Oyetayo (2004) also reported improved weight gain and food intake on rats challenged by enterotoxigenic *E. coli* and given *Lactobacillus achidopillus* probiotics.

Total fecal lactic acid bacteria (LAB): Total fecal lactic acid bacteria (LAB) reflect total LAB in the digestive system of rats. Adding encapsulated *Lactobacillus casei* probiotics in the diet may improve total fecal LAB. The observation on total fecal LAB, which was conducted on day 0, 3 and 7, was presented in Fig. 1.

Mean total LAB in the beginning of observation was log 8.6-9.0 with no significant difference between groups. Total LAB increased on day 3 in all groups, except Group B. Total LAB of Group B was significantly ($p<0.05$) the lowest among all groups. The consistent decrease in total LAB was seen in Group B since Group B was not challenged by *E. coli* and given probiotics in the diet. Total LAB was detected in the fecal of Group B since it was common bacteria in the digestive system. On day 7, total LAB decreased but total LAB in group with probiotics was still higher. This showed that encapsulated *Lacobacillus casei* had potential to increase total LAB in digestive system, even though it was not significantly different. Encapsulation process is able to protect *Lactobacillus casei* bacteria from damage due to extreme circumstance in the digestive system. Moreover, *Lactobacillus casei* is known as having good resistance to gastric acid and bile salt. Salminen *et al*. (1998) mentioned that *L. casei* Shirota strain is resistant to bile salt and stable to gastric acid. Furthermore, Matsumoto (2006) also stated that *L. casei* has good resistance to gastric acid and bile salt so that it can reach intestine alive. Study conducted by Kusumawati *et al*. (2008) found that LAB from lactobacillus genus can grow in digestive system to increase population of beneficial bacteria. The mechanism is by changing pH in intestine which inhibit pathogen growth or in other words, beneficial for LAB growth (Guarner *et al*. 2011).

Total fecal *E. coli*: Total fecal *E. coli* reflect total *E. coli* in digestive system of the rats. Adding encapsulated *Lactobacillus casei* in the diet was expected to reduce *E. coli* in digestive system which was reflected in total fecal *E. coli*. Observation on total fecal *E. coli* was conducted on day 0, 3 and 7 and presented in Fig. 2.

Mean total *E. coli* in the beginning of observation was log 3.9-4.8 and there was not significant difference between groups. On day 3, total *E. coli* increased in Group B, C, D with the highest number in Group B. This was caused by *E. coli* exposure in Group B so that it increased population of *E. coli* in digestive system. Total *E. coli* decreased in all groups on day 7 with lower number in group with probiotics compared to group infected by *E. coli* but not given probiotics. This showed that encapsulated *Lacobacillus casei* had potential to suppress growth of total *E. coli* in digestive system even though it was not significantly different. Probiotics can prevent enteric bacterial infection by competing with pathogen to bind with epithelial cell and improve both specific and non specific immunity response (Reid *et al*., 2003; Zanini *et al*., 2007; Allen *et al*., 2010). Furthermore,

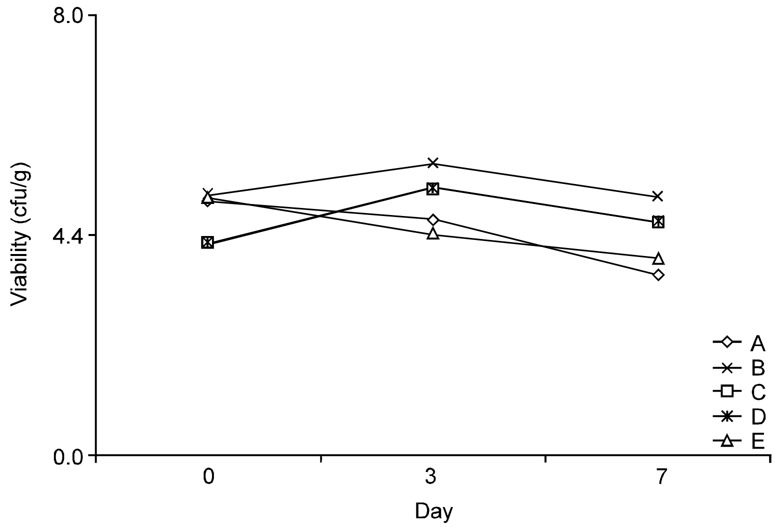

Fig. 2: Total fecal *E. coli* in rats during treatment. ◆: without *E. coli*/without probiotics, x : with *E. coli*/without probiotics, ■: without *E. coli*/with probiotics, *: with *E. coli*/with probiotics, ▲: with *E. coli* and probiotics simultaneously

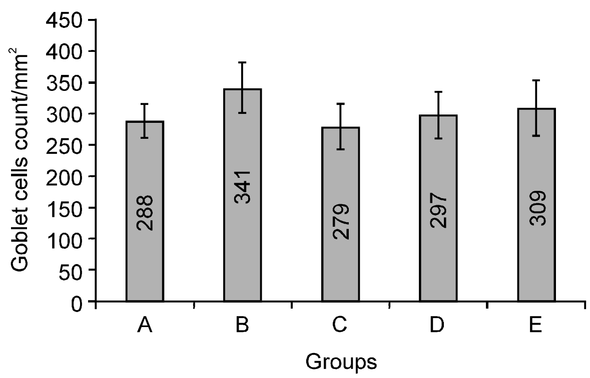

Fig. 3: Total goblet cell in treatment groups, A: with *E. coli*/without probiotics, B: with *E. coli*/without probiotics, C: without *E. coli*/with probiotics, D: with *E. coli*/with probiotics, E: with *E. coli* and probiotics simultaneously

protection from probiotics can inhibit pathogen bacteria since probiotics can create unpleasant environment for pathogen bacteria and compete with the substrate (Olivare *et al*., 2006). *Lactobacillus sp.* genus LAB is known as probiotics with beneficial effect on health, particularly intestine. The reason is that this bacteria has capability to adhere and colonize in the intestine wall (Boekhorst *et al*., 2006). This mechanism may reduce total *E. coli* on rats given encapsulated *Lactobacillus casei*.

Goblet cell: Goblet cell calculation in ileum is related to its function to secrete mucus. Goblet cell can secrete mucus and endocrine cells whose role secreting gastrointestinal hormone to the circulation (Johnson, 2003). Mucus gives additional immunity to pathogen microorganism together with saliva, gastric acid and intestinal peristalsis and proteolysis (Eveline *et al*., 2009). Mucus of goblet cell has many functions, such as protecting from shear stress and chemical hazard (Bowen, 1998). Increasing mucus secretion indicates irritation or infection in the intestine so that the health of intestine is declining. Therefore, goblet cell analysis is needed as indication of probiotic effectiveness. Total goblet cell in each group was presented in Fig. 3, while description of ileum villi section was presented in Fig. 4. Result of ANOVA showed that there was no significant difference on mean total goblet cell between treatments. Mean total goblet cell in Group C was the lowest among other groups, which was 279 cell/mm^2. This indicated that encapsulated *Lactobacillus casei* was safe to consume since there was no excessive irritation compared to Group A. Meanwhile, the highest mean total goblet cell was found in Group B, which was 341 cell/mm^2. This high total goblet cell was due to *E. coli* infection which increased goblet cell proliferation.

Goblet cell can secrete mucus, a thick liquid made from big amount of glycosylated protein (Bowen, 1998). Mucus is one of the products of intestinal secretion with role to balance function of normal digestive system (Heneghan, 1988). Mucus also gives some ecological advantage to intestinal bacteria. Change in goblet cell function and chemical composition on intestinal mucosa indicate abnormality in the intestine detected through big response of luminal, such as change in normal microbiota (Deplancke and Gaskins, 2001). In groups infected by *E. coli* and given probiotics (Group D and E), total goblet cell were lower than group infected by *E. coli* but no probiotics, namely 297 cell/mm^2 and 309 cell/mm^2, respectively. This means that encapsulated

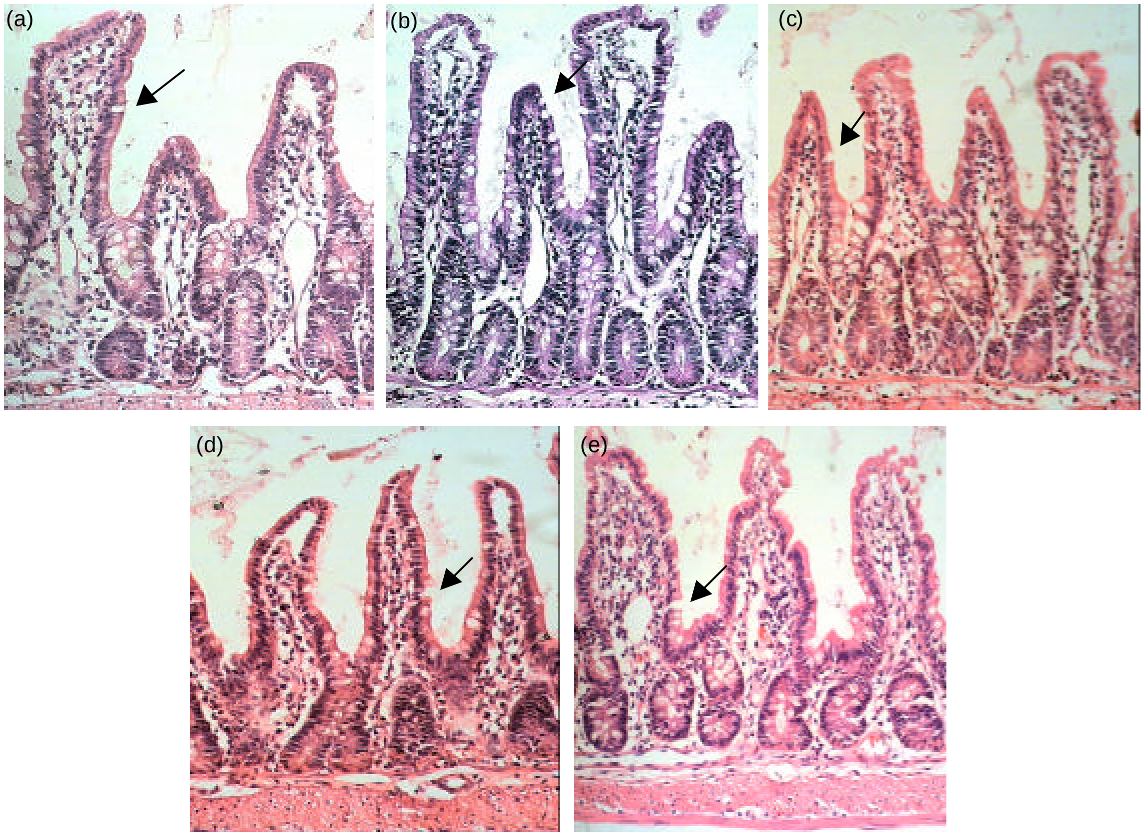

Fig. 4: Ileum villi section, goblet cell was marked with arrow (). A: Without *E. coli*/without probiotics, B: With *E. coli*/without probiotics, C: Without *E. coli*/with probiotics, D: With *E. coli*/with probiotics, E: With *E. coli* and probiotics simultaneously. 200 times magnification

Lactobacillus casei has potential to reduce total goblet cell in ileum of rats infected by *E. coli* even though it was not significantly different.

Conclusions: Encapsulated *Lactobacillus casei* had potential to improve FCE, total fecal LAB and reduce total *E. coli* even though it was not significantly different. This probiotics was also safe to consume and had potential to reduce total goblet cell in ileum of rats infected by *E. coli* even though it was not significantly different.

Recommendations: Recommendation for further study is analyzing the effectiveness with longer treatment. Moreover, the probiotics should be administered in higher dosage.

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