

POTENTIAL OF INDIGENOUS PROBIOTIC *Lactobacillus plantarum* Dad 13 AS ANTI-DIARRHEA AND IMMUNO-MODULATOR

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Abstract

The purpose of the research were to study the effectivity of selected indigenous probiotic *Lactobacillus plantarum* Dad 13 against enteropathogenic *E. coli* causing diarrhea in vivo as well as sinbiotic immunomodulatory properties of purple sweet potato extract probiotic yogurt on blood and liver MDA levels in albino Norway rats (*Rattus norvegicus*) Sprague dawley strain. Unidirection factorial completely randomized design was applied during study with several variables: purple sweet potato yogurt without probiotics addition (Po) and with probiotic addition (Pi), where the latter was conducted on two groups of Sprague Dawley rats treated with or without EPEC ATCC 35218 enteropathogenic *Escherichia coli* (E1 or Eo, respectively). Results showed that there was interaction between indigenous probiotic addition in purple sweet potato yogurt and EPEC ATCC 35218 on faecal water content after 1 week treatment with EPEC ATCC 35218, cecum water content, and MDA level of blood and liver of experimental animals at the end of the study. Besides, culture of *Lactobacillus plantarum* Dad 13 added to purple sweet potato extract yogurt was able to provide preventive health effects as anti-diarrhea and immunomodulatory agent.

Keywords: Probiotic, Anti-diarrhea, Immuno-modulator

Abbreviations:

PSY (Po): Purple sweet potato yoghurt without probiotic (control)

PSYP (Pi): Purple sweet potato yoghurt with probiotic

1. Introductions

Probiotic is living microorganism wherein consumed in adequate amount remain alive inside intestinal gut providing health benefit for body through microbiota balance [1]. [20] mentioned that probiotic bacteria was able to increase immune system and posses several health benefits, such as decrease lactose intolerance incidence, anti-hypertension, and both as prevent and therapeutic agent against diarrhea. Probiotic was also reported to be effective to treat diarrhea caused by *E.coli*, both enterotoxigenic (ETEC) [18] and enterohemorrhagic (EHEC) [14]. Such effect was obtained by more than 10^6 CFU/g or 10^6 CFU/ml living bacteria in intestinal tract [24]. Previous research also indicated that attachment and invasion of enteropathogenic bacteria causing diarrhea was prevented in vitro by *Lactobacillus* and *Bifidobacteria* [5]. Codex requirements for minimum living probiotic cell number in fermentation milk was 10^7 CFU/g [2], which expected to anticipate cell number decrease flow through extreme

condition of intestinal tract [23]. Another research reported that lactic acid bacteria (LAB) consumption from *Lactobacillus* groups able to increase cellular and humoral immune system [8].

Several lactic acid bacteria strains with probiotic property has been successfully isolated (local isolate), such as: *Lactobacillus sp* Dad 13 from fermented buffalo milk (buttermilk), *Lactobacillus plantarum* Mut 7 from Indonesian fermented cassava (*gatot*) and *Lactobacillus acidophilus* SNP-2 from breast milk fed-only baby's faecal. Nutrient in intestinal tract was also needed to preserve colony homeostatis. Normal microsystem formed through probiotic colonization was maintained by diet manipulation using prebiotic, probiotic or combination of both factors known as sinbiotic. The benefit of such combination was enhancement of microflora durability for better health benefits.

A research by [17] reported that purple sweet potato extract yogurt was best prepared with commercial and indigenous probiotic culture combination of *Streptococcus thermophilus* FNCC 0040, *Lactobacillus bulgaricus* FNCC0041 and *Lactobacillus plantarum* Dad 13 in 1:1:0.5 ratio, resulting yogurt with pH of 3.78, viskositas of 5.1987 cP, chromatic color of 18.559, titratable acid of 1.2733%, moisture content of 85.2664 %, ash con-

tent of 0.8041%, reducing sugar of 3.3278%, soluble protein of 1.4782%, fat content of 0.08%, and anthocyanin content of 8.5315%. Organoleptic score for the yogurt for its appearance, taste, and aroma were 2.80; 4.05; 3.35 indicating preference and acceptability. After 2 weeks storage, LAB viability of the yogurt was 10^9 CFU/ml with anti-diarrhea property for able to reduce *E.coli* number up to 4 log cycle.

The present study was conducted *in vivo* using experimental animal. Proposed hypothesis was that LAB *Lactobacillus plantarum* Dad 13 supplemented in yogurt prepared using purple sweet potato extract addition able to prevent diarrhea caused by EPEC as well as positively affecting animal immune system through free radical suppression indicated by blood and liver MDA level decrease. The purpose were to study the effectivity of selected indigenous probiotic *Lactobacillus plantarum* Dad 13 against enteropathogenic *E. coli* causing diarrhea *in vivo* as well as sinbiotic immunomodulatory properties of purple sweet potato extract probiotic yogurt on blood and liver MDA levels in white rats albino Norway rats (*Rattus norvegicus*) Sprague dawley strain..

2. Research and Method

2.1. Material and instrument

Material used were purple sweet potato (*Ipomoea batatas* L) obtained from local market in Sukoharjo, lactic acid bacteria culture of FNCC (Food and Nutrition Culture Collection) provided by Food and Nutrition Laboratory of Inter-university Center Universitas Gadjah Mada Yogyakarta. Culture in slant agar contained *Streptococcus thermophilus* FNCC 0040 and *Lactobacillus bulgaricus* FNCC 0041, and selected indigenous probiotic lactic acid bacteria of *Lactobacillus plantarum* Dad 13. MRS (de Mann Rogossa Sharp) Agar/Broth media was used for maintenance. Materials used in yogurt preparation were skim milk and sugar. Others were chemical for analysis of erythrocytes, leukocytes and MDA, alcohol 70 %, spiritus, and distilled water obtained from Biology, Chemical, and Microbiology Laboratory of Agriculture Department of Universitas Veteran Bangun Nusantara and Food and Nutrition Laboratory of Inter-university Center Universitas Gadjah Mada Yogyakarta

Instrument used during research were: digital scale (Sartorius), oven (Binder), refrigerator (Nasional), incubator (Inko), autoclave (All America), micropipette (Gilson), Juicer (Nasional), Tip and glass laboratory equipment such as test tube (Pyrex), petridish (Anumbra), beaker Glass, erlenmeyer, pipette (Pyrex) etc.

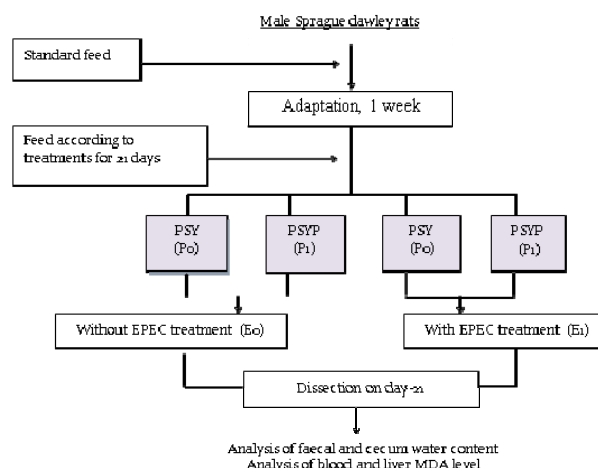
2.2. Research Method

Research diagram was presented in Figure 1.

2.3 Preparation of purple sweet potato extract

Preparation was conducted based on previous study by [17]. Sweet potatoes were diced (5×5 cm), blanched at 100°C for 2 minutes, grounded using fruit juicer, squeezed and filtered. After stand for 30 minutes to 24 hours at 4°C, filtrate was separated to be used in yogurt preparation-Tables

Figure 1. In Vivo Analysis on the effectivity of probiotic



bacteria added on purple sweet potato extract yogurt for diarrhea prevention and imunomodulator

2.4. Preparation of Starter

Three tube of sterile 5 ml MRS broth was prepared; each tube was inoculated with slant culture of *Lactobacillus bulgaricus* FNCC 0041, *Streptococcus thermophilus* FNCC 0040, *Lactobacillus plantarum* Dad13, prior to 24 hours incubation at 36°C. Starter culture was prepared by inoculating 0.1 ml of those cultures into 5 ml sterile skim milk, then incubated at 43°C for 7-8 hours or 36°C for 24 hours.

2.5. Preparation of yogurt using selected formulation

Fresh milk, skim milk (5% w/v) and purple sweet potato extract (10% v/v) were mixed and pasteurized at 72°C for 15 minutes. After cooling into 40-45°C, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were aseptically inoculated together with selected indigenous probiotic *Lactobacillus plantarum* Dad 13 in 1:1:0.5 ratio in 5 % from total volume then homogenized. Inoculated mixture of milk and purple sweet potato extract was put into sterile bottle and incubated at 40°C for 17 hours to obtain purple sweet potato extract yogurt.

2.6. Animal experiment

Factorial completely randomized design was applied in the animal experiment using healthy 2 months-old male Sprague Dawley with body weight 120-130 g provided by National Food and Drug Control Agency (Badan POM RI). The animals were housed individually using 17.5×23.5×17.5 cm³ cage while room temperature was controled in 23-24°C range [15]. Feed was given daily at 06.00-07.00 a.m., 20 g for each animal, drinking water was given *ad libitum*. Feed leftover was collected and weighed to measure daily consumption. Every three days, body weight was measured and cages were cleaned.

Basal feed composition was prepared according to [3] standard, consisted of corn starch, casein (feed protein standard of 10%), corn oil as fat source, mineral mix, CMC as fiber source, and vitamin mix of A, B₁, B₂, B₃, B₆, B₁₂, C, D₃, E and Ca-Pantotenat. Commercial water was used as drinking water.

2.7. Treatment given to animals and sampling method

Rats were divided into 4 groups fed using standard feed. Purple sweet potato extract yogurt with and without probiotic was orally administered for 3 weeks (21 days) with approximately 10⁹ CFU/ml LAB population in yogurt for each rats. Infection of 10⁶ CFU/ml EPEC ATCC 35218 in 1ml/150 g rat body weight was carried out through oral administration for 7 days (from day 8 to day 14). Dissection was conducted at day 21 to collect sample for analysis of faecal and cecum water content as well as rat blood and liver MDA (malonaldehyde).

2.8. Observation of diarrhea incidence on EPEC ATCC 35218 - infected rats

Diarrhea incidence in rats was measured through faecal collected at day 14 and cecum water content in the end of study, using moisture content analysis according to [3]

2.9. Analysis of MDA level

Malonaldehyde (MDA) level in blood and liver was conducted using Thiobarbituric acid substances (TBARS) method in Food and Nutrition Laboratory of Inter-university Center Universitas Gadjah Mada Yogyakarta, according to previously decribed method [16], expressed as mmol/l blood and nmol MDA/ g liver.

2.10. Statistical Analysis

Data obtained was analyzed using CRD Factorial-Anova. Treatments with significant effect were further analyzed using Duncan Multiple Range analysis Construction of references

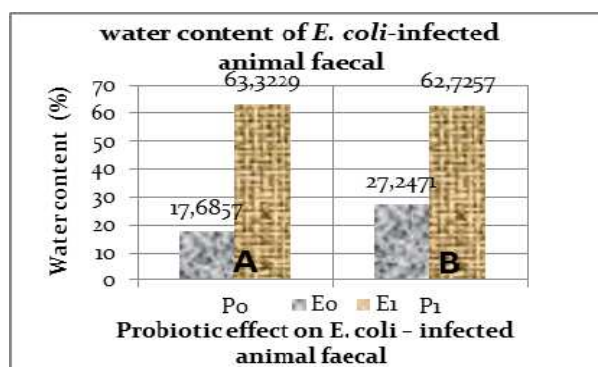
3. Results and Discussion

3.1. Water content of animal faecal after 1 week EPEC ATCC 35218 infection

Statistic analysis indicated that there was interaction between *E.coli* EPEC ATCC 35218 infection and yogurt consumption prepared using indigenous probiotic with water content of animal faecal. Interaction histogram was presented in Figure 2.

Below figure showed faecal water content of 4 groups animal after 1 week infection; histogram A for groups infected and uninfected with EPEC ATCC 35218 (E₀ and E₁) treated with purple sweet potato extract yogurt without probiotic (P₀), while histogram B for infected and uninfected groups treated with probiotic - added yogurt (P₁). Figure 2 showed that in A groups, faecal water content of uninfected rats treated with purple sweet potato extract yogurt without probiotic (E₀P₀) was 17.6857% or lower than uninfected rats treated with yogurt with probiotic (E₀P₁) from group B of 27.2471%, indicating normal range of faecal water content below 60%. Whereas for infected groups, faecal water content of rats treated with yogurt without probiotic (E₁P₀) was 63.3229%, higher than those treated with yogurt with probiotic (E₁P₁) of 62.7257%. *E. coli* infection was shown to increase faecal water content of rats, both without probiotic (E₁P₀) and with probiotic (E₁P₁) above 60%, indicated diarrhea incidence.

Figure 2 Water content of EPEC ATCC 35218 - infected

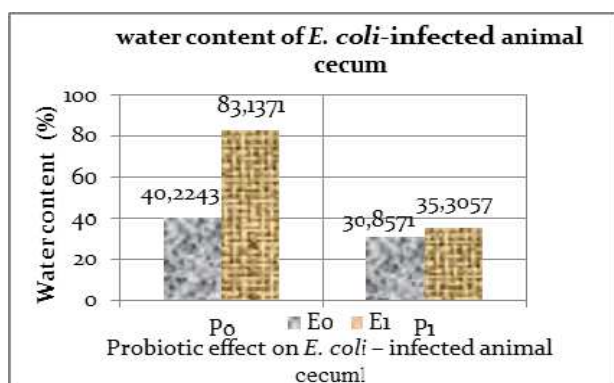


and uninfected animals faecal indicating probiotic consumption effect

Faecal water content of rat group with E₁P₁ treatment was lower than E₁P₀ group, presumably

from protection mechanism of probiotic in purple sweet potato yogurt against EPEC ATCC 35218. [6] mentioned that attachment competition in bonding site and nutrition, immunomodulatory effect, and anti-pathogen compound secretion were among probiotic protection mechanism against pathogenic bacteria. The minimum difference between E1P1 and E1Po group was probably caused by limited probiotic protection in 1 week after EPEC ATCC 35218 infection

Figure 3. Water content of EPEC ATCC 35218 –



infected and uninfected animals cecum indicating probiotic consumption effect

3.2. Water content of animal cecum with EPEC ATCC 35218 infection

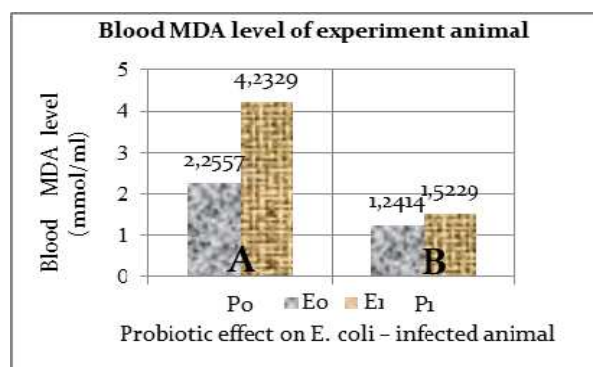
The *cecum* or also spelled caecum, is a pouch or large tubelike structure in the lower abdominal, situated between small and large intestine. Rat's cecum is the place for nutrition fermentation by intestinal microflora [13]. Statistical analysis indicated interaction between *E.coli* infection and consumption of yogurt prepared using indigenous probiotic with water content of animal cecum.

In group A, cecum water content of *E.coli*-uninfected rats administered with purple sweet potato extract yogurt without probiotic (EoPo) was 40.2243%, higher than water content of uninfected rats treated with yogurt with probiotic (EoP1) in group B (30.8571%). As for EPEC ATCC 35218-infected rats, cecum water content of rats treated with purple sweet potato extract yogurt without probiotic (E1Po) was 83.1371%, much higher than those treated with purple sweet potato extract yogurt with probiotic (E1P1) of 35.3057%.

Apparently, diarrhea incidence was occurred due to *E. coli* infection in E1Po and E1P1 rats group. However, E1P1 rats had lower cecum water content than E1Po, indicated probiotic ability to suppress *E.coli* population in E1P1 rat cecum. The

results were comparable to [14] study mentioned that probiotic bacteria suppressed *Escherichia coli* enterohemorrhagic (EHEC) colonization. Besides, *Lacobacillus plantarum* Dad 13 used in purple sweet potato extract yogurt to treat E1P1 group were able to produce anti-microbial compounds. As noted by [22] [9], *E. coli* suppression by *L. plantarum* was due to production of lactic acid with bactericidal and bacteriocin property.

Figure 4. Effect of probiotic consumption on



blood MDA level of EPEC ATCC 35218 – infected and uninfected animals

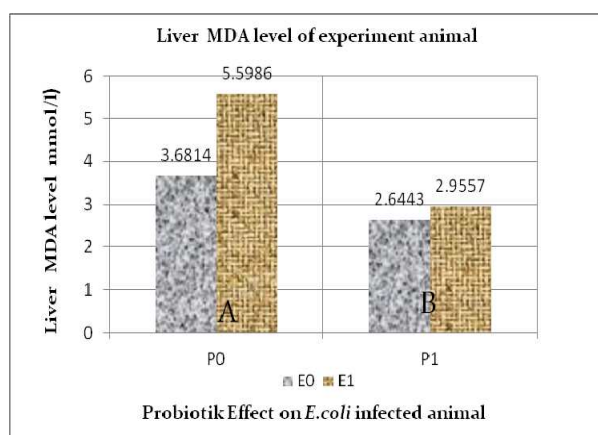
3.3. Blood MDA level of EPEC ATCC 35218-infected animals

MDA (malonaldehyde) is lipid peroxidation product commonly used as tissue and cell oxidative stress indicator [12]. Oxidative stress reflected oxidative damage due to imbalance amount between free radical and antioxidant. For *E. coli* – uninfected rats, blood MDA level of rats administered with purple sweet potato extract yogurt without probiotic (EoPo) was 2.2557 mmol/ml, which was higher than those treated with yogurt with probiotic (EoP1) of 1.2414 mmol/ml. While for infected rats, Figure 4 showed that blood MDA treated with purple sweet potato extract yogurt without probiotic (E1Po) was 4.2329 mmol/ml, which was higher than those treated using probiotic (E1P1) of 1.5229 mmol/ml.

E. coli infection in E1Po and E1P1 rats group was shown to induce rat diarrhea, promoted immune system disruption and increase oxidative stress. Such indirectionally increase free radical in blood reflected by higher level of malonaldehyde (MDA) compare to uninfected rat. Likewise, infected rat treated with probiotic (E1P1) had lower MDA level compare to those treated with yogurt without probiotic. This was apparently caused by probiotic protection mechanism against pathogenic bacteria, through attachment competition on binding site and nutrient, immunomodulatory

effect and secretion of anti-microbia compound [6]. According to [11] probiotic health effect for the hosts body was carried out through maintain microflora balance in intestinal tract and immun response. The mechanisms were competition with enteropathogenic, induce synthesis of cytokine from erythrocytes, producing toxigenic metabolite such as H₂O₂, butiric acid production able to increase erythrocytes turnover, restore normal microflora during antibiotic therapy, and bacteriocin compound production.

Figure 5. Effect of probiotic consumption on liver



MDA level of EPEC ATCC 35218-infected and uninfected animals

3.4. Liver MDA level of EPEC ATCC 35218-infected animals

Rat exposed with EPEC was known to undergo oxidative stress, indirectionally increased free radical in the body. Free radical increase was indicated by higher MDA (malonaldehyde) level, where both factors were concomitant [4].

Figure 5 showed that in group A, liver MDA level of uninfected rat treated with purple sweet potato extract yogurt without probiotic (EoPo) was 3.6814 mmol/ml or higher than those uninfected rat treated with yogurt with probiotic (EoP1) in group B, with MDA level of 2.6443 mmol/ml. While for *E. coli*-infected rat, liver MDA level of those treated with purple sweet potato extract yogurt without probiotic (E1Po) of 5.5986 mmol/ml was higher than rat treated with purple sweet potato extract yogurt with probiotic (E1P1) of 2.9557 mmol/ml.

Free radical increase stored in liver was indirectionally caused by diarrhea-induced oxidative stress and immune system disorder in E1Po and E1P1 groups. Thus liver MDA level increased with *E. coli* exposure, higher than uninfected rat. However, treatment using purple sweet potato extract

yogurt with probiotic resulting lower liver MDA level compare to those treated with yogurt without probiotic. This was caused by protection mechanism of probiotic bacteria against pathogenic bacteria through pathogen inhibition from nutrition competition, pH decrease and bacteriocin secretion, toxin production and attachment inhibition, virulence property removal, immune system enhancement [7] and also probiotic adhesion to intestinal mucose [19]. [23] noted that several probiotics served as immunomodulatory agent in intestinal tract through up-regulation of anti-inflammatory factors, immunomodulation by suppression of proinflammatory factors, enhancement of immunity, epithelial cell differentiation and proliferation, as well as and promotion of intestinal barrier function.

4. Conclusions

From the research, it was concluded that

(1) After 1 week EPEC ATCC 35218 treatment, there was interaction between the treatment and consumption of indigenous probiotic in purple sweet potato extract yogurt with water content of animal faecal and cecum as well as blood and liver MDA level of the animals at the end of study (2) *Lactobacillus plantarum* Dad 13 culture added to purple sweet potato extract yogurt possessed health effect to prevent diarrhea and serve as immunomodulator.

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