

Study of antidiarrheal and hematology profile of laboratory rat fed with yogurt containing local probiotic and purple sweet potato extract

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Abstract. The aim of this study was to evaluate the effectiveness of local probiotic in yogurt with purple sweet potato extract supplementation on the hematological parameters of albino rats (*Sprague dawley*). The study was conducted using a Completely Randomized Design with 30 rats divided into 6 groups. In group K-, rats were fed with distilled water from day 1 to 21. In group YTP, Rats were fed with yogurt without probiotics from day 1 to 21. YDP group was rats were fed with probiotic yogurt from day 1 to 21. In group YTP+E, rats were fed with yogurt without probiotic from day 1 to 7, interspersed with exposure to enteropathogenic *Escherichia coli* (EPEC) on day 8 to 14. In group YDP+E, rats were fed with probiotic yogurt from day 1 to 7, interspersed by EPEC on day 8 to 24. In group K +, rats were fed with water from day 1 to 7, then fed with EPEC on day 8 to 14, after which water was given back on day 15 to 21. The result showed that probiotic yogurt treatment with supplement of purple sweet potato extract had a significant effect ($P < 0,05$) on feces water content, number of erythrocyte, leucocyte, and hemoglobin. The treatment of YDP had water content in feces 48.422% and the number of erythrocyte, leucocytes, and hemoglobin were $8.578 \times 10^6/\mu\text{l}$, $14.152 \times 10^6/\mu\text{l}$ and 13.98 g / dL respectively.

1. Introduction

Probiotic is a biological microorganism which still survives at the time of reaching the digestive tract, its provides the health benefit to the body through microbiotic balance [1]. According to previous study [2], probiotics are beneficial to increase physical immunity,. and also reported to be able to overcome diarrhea caused by *E.coli*, either enterotoxigenic (ETEC) [3] or enterohemorrhagic (EHEC) [4]. Such effect emerges if a number of biological bacteria reaching the digestive tract are more than 10^6CFU/g or 10^6CFU/ml [3]. The required minimum amount of biological cell of probiotics in yogurt is 10^7CFU/g [5], which is expected to be able to anticipate the decrease in the amount of cell during passing the extreme environment in the digestive tract [6]. The ability of probiotics to protect intestine is assumed through several mechanisms, such as the ability to compete with nutrition in such a way that it inhibits the growth of enteral bacteria in the human digestive tract. Probiotics is also able to compete for the adhesive side of the surface of intestinal cell against the pathogenic bacteria causing the infection of digestive tract in such a way that it can inhibit the adhesion or exclude the pathogenic bacteria or displace the adhesive side of pathogenic bacteria which has previously been adhesive to the intestine [7,8].



Nowadays, experts are intensely promoting the concept of food as medicine. Such concept is a way to optimize functional food in order to overcome diseases. One of the functional food types from own is sweet potato. Sweet potato contains such antioxidants as phenolic acid, anthocyanin, and tocopheryl, which can prevent several diseases [9]. The improvement in the savor of sweet potato in order to maximize its utilization as alternative diet, such as converted into yogurt.

Some local lactic acid bacteria have been isolated such as *Lactobacillus plantarum* Mut 7 isolated from dried cassava fermentation [10], *Lactobacillus plantarum* Dad 13 isolated from buffalo fermented milk [11], and *Lactobacillus acidophilus* SNP-2 and *Lactobacillus acidophilus* SNP-2 which are isolated from the baby's stools consuming breast milk [12].

Probiotic bacteria that given orally has the ability to affect to body metabolic system, including the status of hematology or blood function [13]. There is no information about local probiotic on hematology profile in suppressing the growth of EPEC ATCC 35218. Therefore, this study aims to evaluate the effectiveness of local probiotic in yogurt with purple sweet potato extract supplementation on the hematological parameters of albino rats (*Sprague dawley*).

2. Method

2.1. Materials and Equipment

Materials used in this research were purple sweet potato (*Ipomoea batatas* L) and yogurt starter culture: *Streptococcus thermophilus* FNCC 0040, *Lactobacillus bulgaricus* FNCC 0041 and indigenous probiotic lactic acid bacteria : *Lactobacillus plantarum* Dad13, obtained from Food and Nutrition laboratory on Inter-university UGM Yogyakarta. MRS media (de Mann Rogossa Sharp) Bacteriological Agar/Broth (Oxoid), for Lactic Acid Bacteria culture, Nutrient Broth medium (Merck) for EPEC ATCC 35218 culture. Other materials were skim milk, alcohol 70 %, spiritus, and distilled water obtained from Biology, Chemical, and Microbiology Laboratory of Agriculture Department of Veteran Bangun Nusantara University. Equipment used in this research were glassware (test tube, Beaker glass, Erlenmeyer, and Petridish), autoclave (All America), incubator (Inko), Oven (Binder), and entkas.

2.2. The making of yoghurt with the supplement of sweet potato extract

Fresh milk, skimmed milk (5% b/v), and the sweet potato extract (10% v/v) were pasteurized at the temperature of 72°C for 15 minutes followed with cooling down to the temperature of 40-45°C. Next, it was inoculated with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* along with the indigenus probiotic bacteria with the ratio of 1 : 1 : 0.5 aseptically at the temperature of 43-45°C, as much as 5 % (v/v), and then it was shaken up for becoming homogeneous and incubated at the temperature of 40°C for 17 hours in such a way that yoghurt with the supplement of sweet potato extract is yielded.

2.3. Administration of the Experimental Animal

This research was conducted by using 20 male rats of *Sprague dawley* strain, 5 – 6 weeks old, at 100 – 120 g body weight. The cage used was an individual cage with the size of 17.5 x 23.5 x 17.5 cm without base. The room temperature was adjusted into 23-24°C [14]. The ration was administered as much as 15 g for each rat for each day at 06.00 – 07.00 AM (GMT+7).

The water was provided ad libitum. The rest of the ration was collected on a daily basis in order to be weighed and found out for the consumption of ration by each rat for each day. Weighing the body of the rats was conducted every 3 days. The composition of the basal ration was set up on the basis of the American Institute of Nutrition (AIN)-93 in 1 kg consists of 620.7 g of cornstarch, 140 g of casein, 100 g of sucrose, 40 g of oil, 50 g of fiber, 35 g of mineral, 10 g of vitamin, 1.8 g of L-cysteine, 2.5 g of choline, and 0.008 g of tert-Butylhydroquinone (TBHQ). The experiment flow provided in Figure 1.

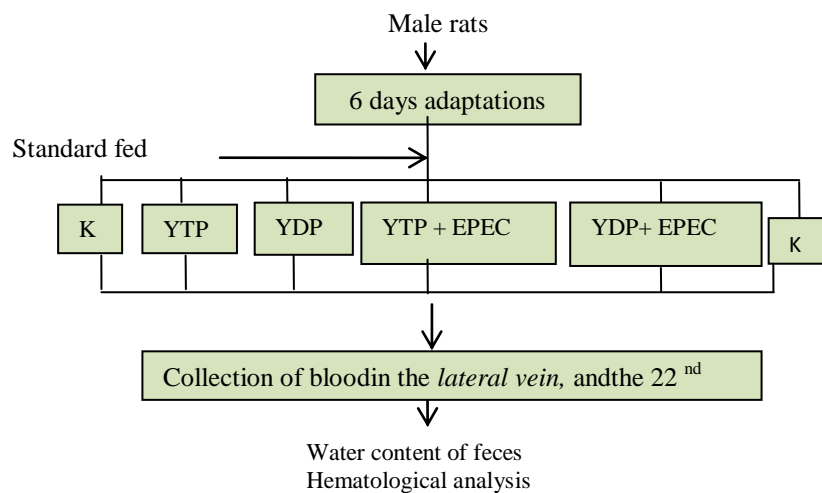


Figure 1. Research Flow chart

Note 1

- Control Rats were fed with distilled water from 1st -21st day
- YTP Rats were fed with yogurt without probiotics from 1st -21st day
- YDP Rats were fed with probiotic yogurt from 1st - 21st day
- YTP +EPEC rats were fed yogurt without probiotic from 1st - 21st day, interspersed by EPEC on 8th– 14th day
- YDP +EPEC = Rats were fed with probiotic yogurt from 1st - 21st day, interspersed EPEC on 8th-14th day
- + Control Rats were fed with water from 1st - 7th day, then fed EPEC on 8th - 14th day, after which water was given back on 15th - 21st day

Note 2

- Probiotics is administered orally as much as 1 mL/day from the 1st day - the 2nd day with the population of LAB 10⁹ CFU/ml by using feeding hose
- Infection by EPEC ATCC 35218 is administered orally as much 1 mL/day from the eighth day to the fourteenth day with the population of EPEC 10⁷ CFU/mL
- The termination of rat was conducted each time against six rats on the twenty second day

2.4. Research Design and Statistical Analysis

This research used the Completely Randomized Design with six treatments, namely K-, YTP, YDP, YTP+E, YDP+E and K+ and 5 repetitions, each consisted of 1 rat. The data were analyzed using ANOVA, any significant treatment followed with DMRT (Duncan's Multiple Range Test) [15]

2.5. Observation Parameters

2.5.1. Fecal water content The water content of animal feces was observed on the 14th day, using the thermogravimetric method [16]

2.5.2. Hematology analysis Hematology analysis included number of erythrocytes, leucocytes, and hemoglobin levels were performed. Hematologic analysis was performed according to [13] methods. Blood samples were collected in the tube containing EDTA. The analysis was performed using Hemavet HV950FS multispecies hematology analyzer.

3. Result and Discussion

3.1. Water content of feces

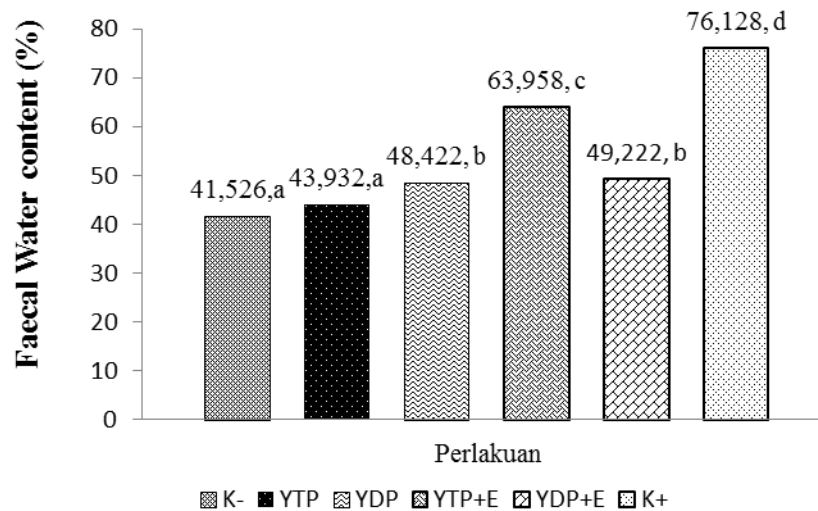


Figure 2. Histograms of fecal water content (%) of experimental animals in various treatments of probiotics yogurt with supplement of purple sweet potato extract and after an exposure to enteropathogenic *Escherichia coli* (EPEC) ATCC35218

The water content of rat's feces is presented in Figure 2. Figure 2 showed that the treatment of probiotic yogurt with supplementary of purple sweet potato extract and after exposure of enteropathogenic *Escherichia* was significant ($P < 0,05$) to the water content of rat's feces. Rats group treated with YTP + E (yogurt without probiotics interspersed and EPEC) and K + (positive control) had high level of fecal water and were classified under diarrhea (above 60%). [17] reported that the normal stool water content of rats was below 60%. The condition of diarrhea in rats was characterized by soft feces and water content above 60%, whereas very severe diarrhea in rats was characterized by liquid feces and water content above 80%

The group of YDP + E rats may have no diarrhea occurrence, although the water content was slightly higher than that of the K-rats, YTP, and YDP. This was possible because of the probiotics in purple sweet potato extract yogurt was able to protect against EPEC bacteria ATCC 35218. According [7] mechanism of probiotic bacteria protection against pathogenic bacteria, among others through attachment competition on bonding and nutrient side, modulate the immune system and secretion of an anti microbial compound

3.2. Blood Test (Hematology)

Blood is an important component because it functions to circulate blood that goes into body as well as produced by body from metabolism process. Hematology value (blood profile) is useful to assess health condition and as baseline or control in research.

3.2.1. Erythrocytes

The number of normal erythrocytes ranges from $6.6-9.0 \times 10^6 / \mu\text{L}$ [18]. Groups of rats in K + and YTP + E had lower erythrocytes ($P < 0.05$) than the rats in K-group, YTP, YDP (Figure 3). EPEC treatment caused cell membrane damage and disrupted the cell wall permeability properties, resulting in leakage of cells and loss of some metabolites, and ended with the reducing of erythrocytes [13]. The

probiotic (YDP) group of rats had the highest erythrocyte ($8,578 \times 10^6 / \mu\text{L}$), this was because probiotic bacteria had bacteriostatic and bactericidal properties by protecting the plasma membrane.

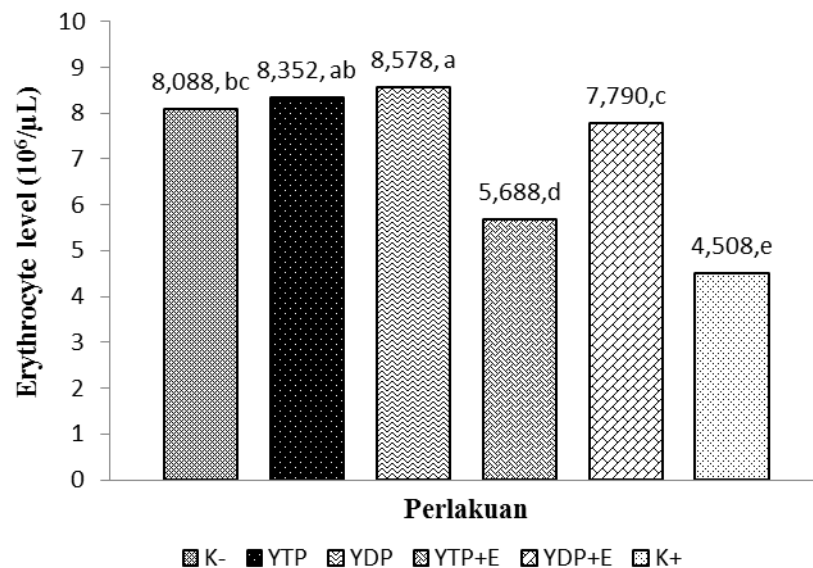


Figure 3. Histogram of erythrocyte level $10^6 / \mu\text{L}$ of experimental animals on various treatments of probiotic yogurt with purple sweet potato extract supplementation and after exposure to enteropathogenic *Escherichia coli* (EPEC) ATCC35218

3.2.2. Leukocytes

Groups K + of rats had significant lower leukocytes ($P < 0.05$) than in the K-group, YTP, YDP, YTP + E and YDP + E (Figure 4). The high number of leukocytes in the YTP and YDP rats when compared to K +, was possible due to the probiotic ability of the immunomodulator, which can boost the immune system [18]. Rats group with YTP treatment could increase the number of probiotics because the presence of prebiotic in the form of purple sweet potato could increase the number of probiotics. [19] reports that probiotic bacteria can attach to the intestinal surface to protect a host from pathogenic bacterial colonization by different mechanisms.

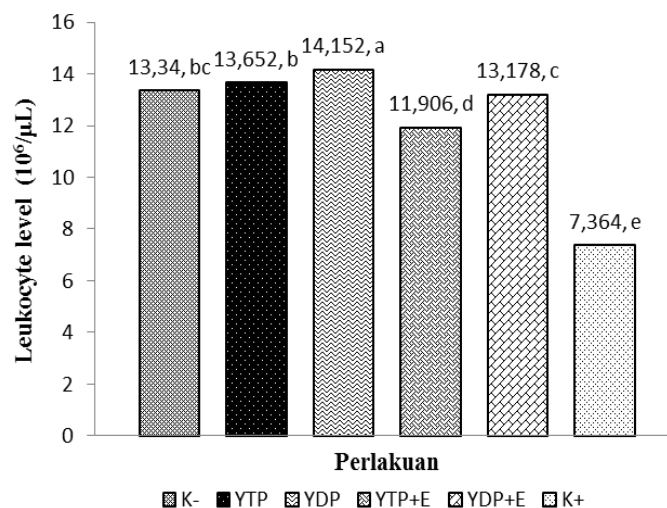


Figure 4. Histogram of leukocyte level $10^6 / \mu\text{L}$ of experimental animals on various treatments of probiotic yogurt with purple sweet potato extract supplementation and after exposure to enteropathogenic *Escherichia coli* (EPEC) ATCC35218

The decrease of the number of leukocytes in the group of rats treated with YTP + E and YDP + E compared to K- was possible injury to the intestinal mucosa due to EPEC infection so that leukocytes were used for body defense. This condition caused the number of leukocytes in the blood circulation to be reduced.

3.2.3. Haemoglobin

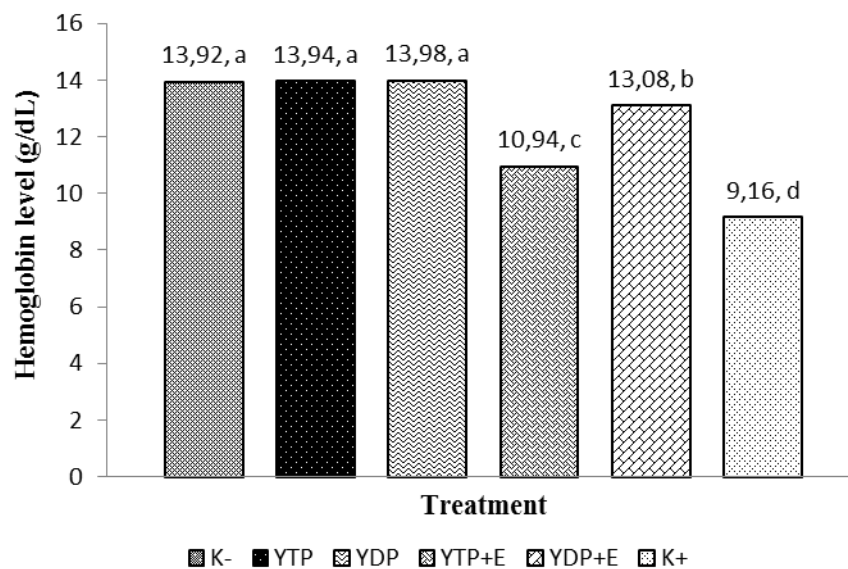


Figure 5. Histogram of hemoglobin level (g/dL) of experimental animals on various treatments of probiotic yogurt with purple sweet potato extract supplementation and after exposure to enteropathogenic *Escherichia coli* (EPEC) ATCC35218

Hemoglobin (Hb) is an erythrocyte pigment consisting of a conjugated complex protein containing iron. Normal hemoglobin level is 12.0-17.5 g/dL. Figure 5 showed that groups of rats treated with K + had lower hemoglobin ($P < 0.05$) than the K-group, YTP, YDP, YTP + E or YDP + E.

According to [13], pathogenic bacteria such as EPEC can damage the permeability of cell membranes and cause cell wall damage, resulting in the release of hemoglobin from cells and causing decreased hemoglobin levels. Group YTP + E rats and YDP + E rats had higher hemoglobin levels than the group K rats. In the group of YTP rats, a prebiotic of purple sweet potato could increase the number of probiotics. Whereas in the group of YDP rats there were probiotics. Probiotics in both groups of rats had the ability to produce antimicrobials which was capable of inhibiting EPEC growth, thereby reducing intestinal epithelial damage.

4. Conclusions

Probiotic yogurt treatment with supplement of purple sweet potato extract had a significant effect ($P < 0.05$) on feces water content, the number of erythrocyte, leucocyte, and hemoglobin. The treatment of YDP rats had water content of feces 48.422%, while number of erythrocyte, leucocytes, and hemoglobin were $8.578 \times 10^6/\mu\text{L}$, $14.152 \times 10^6/\mu\text{L}$ and 13.98 g / dL.

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