International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

Effect of Synbiotic Yogurt Extract of Red Dragon Fruit Peel (*Hylocereus polyrhizus*) with Stevia on Intestinal Histology and Microbiota Experimental Animal

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Abstract**—** *The aim of this research is to find out about the effect of giving synbiotic yoghurt with red dragon fruit peel extract with stevia sweetener in experimental animals. The material for this research was wistar mice (Rattus norvegicus) which were treated with yoghurt made from skim milk and fermented with yogurt starter containing Lactobacillus bulgaricus and Streptococcus thermophilus (1:1), and the addition of 0.5% stevia to synbiotic yoghurt. The experimental research method with the given treatments P1: experimental animal + probiotic yoghurt, P2: experimental animal + synbiotic yoghurt with red dragon fruit peel extract (Hylocereus polyrhizus) 20% (v/v), and P3: experimental animal synbiotic yoghurt with red dragon fruit peel extract (Hylocereus polyrhizus) 20% + 0,5% stevia (v/v). The observed variables were histology profile and gut microbiota of the experimental animal ileum. The administration of yoghurt treatments P1, P2, and P3 increased the villus height and decreased the villus width. The total microbes in all treatments decreased. The total of lactic acid bacteria increased in treatment P1 and P2, then decreased in P3. The research result showed that the administration of probiotic yoghurt, synbiotic yoghurt, and synbiotic yoghurt with the addition of stevia influenced the histology profile and the gut microbiota.*

Keywords**—** *Dragon fruit peel extract, histology, stevia, synbiotic, yoghurt.*

I. INTRODUCTION

Yoghurt is a processed product using fermentation technology with lactic acid as a starter. Starter activity of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in milk works in a mutualistic symbiosis to produce yoghurt (Aswal, *et al*., 2012). One of the efforts to increase the functional properties of yoghurt is by adding prebiotic in the form of fiber. Prebiotic can be the source of energy for intestinal epithelium and substrate for bacteria to produce functional compounds such as vitamins and antioxidants. Yoghurt with a combination of probiotics and prebiotics is called synbiotic yogurt. Synbiotic yoghurt consists of bacteria (probiotics) and indigestible carbohydrates (prebiotics) to stimulate the growth of beneficial bacteria. Synbiotic product is expected to increase probiotics' vitality and as an energy source for microbes in the digestive tract.

Prebiotic in synbiotic yoghurt must have optimum vitality to stay alive, grow, and be active in the digestive tract. To be resistant to acid, bile salt. To be able to grow fast and attach (colonizing) to the wall of digestive salt. To be able to prevent and kill the presence of pathogenic bacteria (Widodo, 2003). Good digestive condition can be seen from the lactic acid bacteriaance of the microbiota and intestinal histology. The application of synbiotic yogurt to the experimental animal (mice) can increase the function of the intestinal barrier to maintain intestinal permeability and prevent intestinal villus damage. Good intestinal conditions can maximalize the absorption of the nutrient which affects the health of the host. Consumption of prebiotic bacteria can boost the immune system and has a health effect on the host in both human and experimental animals like mice (Tari, *et al*., 2020).

Synbiotic yoghurt with red dragon fruit peel extract (*Hylocereus polyrhizus*) has an unfavorable aftertaste (Waladi, *et al*., 2015). Sweetener is added to overcome the aftertaste. It is important to note the caloric value when the sweetener is added to yoghurt, as not to influence the functional properties of yoghurt. It is advised to use low-calorie sweeteners. Stevia is a low-calorie sweetener that remains stable when combined with foods that are processed at high temperatures, acidic, and fermented products (Argawal, *et al*., 2010). Stevia is better used as a natural sweetener than fructose syrup and honey super in fortified synbiotic yogurt with evaporated red dragon fruit waste extract seen from average pH value, total acid, fat level, and protein content (Sawitri, *et al*., 2020). Stevia has several properties, such as anti-bacterial, anti-inflammation, anti-hyperglycaemic, anti-hypertensive, and anti-tumor activities. Furthermore, several studies have shown that stevia exerts anti-microbial, anti-inflammatory, and antioxidant effects both in vivo and in vitro (Moselhy, *et al*., 2016; Roberts and Munro, 2009; Casas, *et al*., 2019; Boonkaewwan and Burodom, 2013). Stevia contains anti-microbial that can be used to manipulate gut microbiota (Boling, *et al*., 2020). The utilization of stevia 400 mg/kg bb can fix duodenum, jejunum, and ileum morphology (Mehmood, *et al*., 2019).

Based on the explanation above, the fiber content and the antioxidant of the red dragon fruit peel waste (*Hylocereus polyrhizus*) with the addition of natural sweetener (stevia) in synbiotic yoghurt with red dragon fruit peel extract (*Hylocereus polyrhizus*) has the potential to increase the health of the digestive tract. So, it needs to be researched to find out

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International Research Journal of Advanced Engineering and Science
ISSN (Online): 2455-9024

about the intestinal histology and microbes of the experimental animal.

II. Materials and Methods

A. Materials

The experimental animal was 3-month-old male wistar mice (*Rattus norvegicus*) weighing 150-180 g which were given yoghurt probiotic treatment (P1), fortified synbiotic yoghurt with red dragon fruit peel extract 20% (P2), and fortified synbiotic yoghurt with red dragon fruit peel extract 20% with stevia sweetener 0,5% (P3) where each treatment group consisted of 5 experimental animals. The experimental animals were adapted for 7 days with feed and drink aqua by *ad libitum*. The equipment used during the keeping, such as group cage, feeding, and drinking place.

Probiotic yoghurt (P1) was made from skim milk (10%) with the addition of aquades, as for synbiotic yoghurt (P2 and P3) was added the red dragon fruit peel extract 20%. All samples were pasteurized at 72℃ for 15 minutes, then the temperature was lowered to 42℃, and the mixture was well stirred after inoculating starter yogurt (3%), contain *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (1:1). Incubation at room temperature (25-28℃) for 24 hours. In treatment P3, the stevia 0,5% was added. Yoghurt was given to the experimental animal by ad libitum for 30 days.

B. Analyzed Procedure

The sampling of the intestinal samples and excreta was done the pre-post test. The experimental animal was euthanized using chloroform, before treatment at the start and after treatment at the end for 30 days. Histological observation of small intestine samples using haematoxylin - eosin staining method (Iji, *et al*., 2001). The small intestine segment prepared for histology was the ileum which span was folded to form parallel loops. The tissues were fixed in 10% neutral buffered formalin over 24 h. To prepare the hematoxylin-eosin preparation, every piece of the tissue samples was hydrated through a series of alcohol with increasing concentrations. The samples were transferred one by one in every alcohol concentration and left to soak in every alcohol concentration for about 10 seconds. The sample was put in xytol and finally dipped in paraffin. By using a microtome, the sample was thinly sliced for further haematoxilyn - eosin painting. Slides were viewed on an Olympus 4X microscope and digitized using image soft-ware (Optilab Viewer). Images were viewed with 100x magnification to measure the villus height and villus width (Image Raster 3).

The testing procedure of TPC (Total Plate Count) was conducted using Plate Count Agar (PCA) and De Man, Rogosa, and Sharpe Agar (MRSA) media by pour plate (Nuriyasa, *et al*., 2020; Putera, *et al*., 2012). The excreta is taken from the ileum of the rat, placed in a plastic clip, then taken to the laboratory for testing. Sterilize the equipment and agar using an autoclave at temperature of 121℃ for 15 minutes, while sterilizing PCA (Plate Count Agar), MRSA (De Man, Rogosa, and Sharpe Agar) and Peptone water (0,1%). The sample dilution is carried out by placing 1 g of excreta into a test tube containing 9 ml of peptone water, shaking it so that it is homogeneous, the dilution is continued until the dilution reaches 10^8. To find out the total microbes and the total lactic acid bacteria by using the Pour Plate Method. Samples were pipette 1 ml from each dilution 10^6, 10^7 to 10^8 and put into sterile Petri dishes. Added 12 ml to 15 ml of sterile PCA for testing total microbe and MRSA for testing lactic acid bacteria. The total plate count (TPC) used PCA media, which had been cooled in a water bath to reach a temperature of 45℃, into each petri which already contained 1 ml of sample. For the sample and media to be completely mixed, the plates were rotated following the number 8 rotation. After the media became solid, then it was incubated in the best position in the incubator for 24 hours at 37℃. The total colony per gram sample can be calculated using the colony counter (Quebec DC-3).

III. Result and Discussion

A. Ileum Histology of Experimental Animal

The villus height of the experimental animal increased after giving the experimental animal yoghurt for 30 minutes (Table I).

TABLE I. Average Height and Width of Experimental Animal Villus.

Treatment	Villus height (µm)		Villus width (µm)	
	Pre	Post	Pre	Post
P1	192,63	204,42	49.40	33.97
P2	150,34	208,26	45.37	38.02
P3	163,72	265,02	48.65	45.13

Results show that yoghurt can increase the villus height to better absorb nutrients. Fermented milk can influence the histology structure of the small intestine by increased the villus height (Allori, *et al*., 2000). The highest height and width of the villus was in the experimental animal that was given synbiotic yoghurt with red dragon fruit peel extract (*Hylocereus polyrhizus*) and stevia sweetener. Synbiotic yoghurt was composed of probiotics in the form of lactic acid bacteria and prebiotics which are generally in the form of fiber, which in this research was from red dragon fruit peel extract (*Hylocereus polyrhizus*). Red dragon fruit peel extract contains pectin which is classified as a polysaccharide that can function as prebiotics and probiotics in synbiotics products. Pectin interacts with differentiated intestinal cells and stimulates the proliferation of undifferentiated intestinal cells (Nishida, *et al*., 2015). The proliferation of the intestinal cells influenced the height and width of the villus of the experimental animal. The increasing of the height and width of the villus can provide a larger surface area to absorb nutrients and leads to better bowel performance (Mile, *et al*., 2006; Awad, *et al*., 2009).

Synbiotic yoghurt contains many active materials which can be beneficial for the health of the host, one of them is by improving the health of the digestive tract. An increase in the villus height and a decrease in the villus width indicate that the villus was not swollen (inflammation), it indicates that there was no damage to the villus. Synbiotic yoghurt can increase the height and width of the villus, it can increase the function

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of the intestinal barrier to maintain permeability and to prevent intestinal damage (Miller, *et al*., 2021).

The administration of the synbiotic yoghurt with the addition of stevia sweetener showed the highest villus height compared to other experimental animal samples in this research. The increase of the villus height in the experimental animal intestine is in line with the research by Mehmood, *et al*., (2019) which is the administration of stevia extract 400 mg/kg bb shows the improvement of the duodenum, jejunum, and ileum morphology. Steviol glycosides in stevia contain various phytochemical compounds. The phytochemicals are austroinullin, -carotenoids, dulcoside, nilacin, rebaudi oxides, riboflavin, steviol, stevioside, and thiamine (Crammer and Ikan, 1986). Phytochemical can maintain or increase the normal intestinal morphology, it can increase the villus height thus increasing the absorption of the nutrients on the intestinal surface (Tabatabaei, 2016). Steviol glycoside is non-toxic and does not induce hypotension, cardiotonic, anti-carcinogenic and anti-inflammatory, (Mathur, *et al*., 2017).

B. Intestinal Microbiota

TABLE II. Average of the total microbes and the total lactic acid bacteria of the experimental animal ileum.

Treatment	Total Mikroba (x10^9 CFU/ml)		Total LACTIC ACID BACTERIA (x10^9 CFU/ml)	
	Pre	Post	Pre	Post
P1	70.4	27.4	0.96	1.21
P2	98.6	57.6	2.08	2.69
P3	145,2	55.1	1.73	0.25

Based on the observation of table II, yoghurt can decrease the total microbes in the experimental animal intestine. The decrease of the total microbes can be interpreted to reduce the number of pathogenic bacteria in the small intestine. Yoghurt contains lactic acid bacteria which can produce bacteriocins as antimicrobial. The effect of antimicrobial of the lactic acid bacteria is formed by producing several substances such as organic acid (lactic, acetic, propionic acids), carbon dioxide, hydrogen peroxide, diacetyl, low molecular weight antimicrobial substances, and bacteriocins (Ouwehand and Vesterlund, 2004). These antimicrobial substances can inhibit cell metabolism, protein synthesis, the permeability of the cell membrane to damage the nucleic acids and proteins of pathogenic bacteria, thereby triggering overall cell damage. Some beneficial effects of probiotic microbes in the gut are: supporting intestinal mucosal function, producing antimicrobial substances (for example bacteriocins), reducing pH, colonialization of the intestinal epithelial surface, immunostimulating effect, anti-inflammatory properties (Kumar, *et al*., 2015).

Synbiotic yoghurt contains more bioactive components, because of the addition of prebiotic materials in it. Dragon fruit peel extract contains bioactive components such as betanin, phyllotactic, hylocerenin, betacyanin, pectin, triterpenoids, and steroids (Luo, *et al*., 2014). These components function as anti-inflammation, antioxidants, and antimicrobial. Steroids and triterpenoids compound in red dragon fruit peel acts as antimicrobial (Manihuruk, *et al*., 2017). Besides that, commercial stevia added to synbiotic yoghurt contains various active compounds that act as antimicrobial. Stevia contains stevioside (rebausida) which has antimicrobial and antifungal properties (Atteh, *et al*., 2008).

The decreasing of the total microbes is in line with the increase of the total lactic acid bacteria in the experimental animal ileum after it is given yoghurt according to the treatment. Prebiotics that are contained in yoghurt can increase the quality of the live prebiotics, which is lactic acid bacteria. Synbiotic yoghurt is composed of bacteria (probiotics) and indigestible carbohydrates (prebiotics) to induce the growth of beneficial bacteria (Khurana and Kanawjia, 2007). The increasing of the lactic acid and ileum can prevent the damage to the ileum due to pathogenic bacteria by producing bacteriocin and prevent colonialization of the pathogenic bacteria in the ileum. Probiotics can inhibit the growth of pathogenic bacteria through the production of bacteriocin and compete with pathogens for the binding to epithelial cells (De Roos and Katan, 2000). This prevents the colonialization of the pathogen to the ileum, to minimize the damage to the intestinal villus.

The administration of the synbiotic yoghurt with red dragon fruit peel extract (*Hylocereus polyrhizus*) and stevia sweetener in the experimental animal show the decreasing of the total lactic acid bacteria in the ileum. The decrease is thought to be because of many lactic acid bacteria attached to the villus, so it does not include in the excreta. Probiotic bacteria can be attached to the surface of the intestine to increase the digestive tract defense.

IV. CONCLUSION

In conclusion, synbiotic yoghurt with stevia sweetener 0,5% can increase the height and width of the villus, and reduce the number of microbes in the experimental animal ileum. Total lactic acid bacteria decrease in the ileum, which can be interpreted that are more lactic acid bacteria attached to the intestinal villus.

ACKNOWLEDGMENT

This research is part of the Doctoral Grant Program of Head Lecturer of Universitas Brawijaya 2021.

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