

Synbiotic Purple Sweet Potato Yogurt Ameliorate Lipid Metabolism in High Fat Diet Mice Model

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Dyslipidemia has high prevalence worldwide. Unhealthy diet such as high calories and high fat contribute to dyslipidemia. Purple sweet potato yogurt is a synbiotic product containing combination of probiotic fermentation and natural ingredient (prebiotic). Probiotics give beneficial effect to intestinal function. Purple sweet potato serves as prebiotic, also contains anthocyanin as antioxidant. This experimental study aimed to identify the effect of purple sweet potato yogurt consumption towards lactic acid bacteria colony in the intestine, lipid profile (total cholesterol, triglyceride, LDL), body weight, visceral fats, and liver weight of male mice model (Balb-c) with high fat diet. Total of 30 male mice were divided into six groups with different treatment on its diet. Lipid profiles were investigated using enzymatic colorimetric assay. Statistical data was analyzed using one-way ANOVA and post hoc tukey multiple comparison test. Result shown that in the group with administration of 0.3 mL of purple sweet potato yogurt had significantly increase the growth of lactic acid bacteria in the intestine ($p < 0.0001$). The same group also shown favorable outcome in total cholesterol, triglyceride, LDL level, visceral fat and liver weight. Purple sweet potato yogurt has potential protective effect in lipid metabolism under high fat diet condition. Thus, further study is needed to explore more detail on its mechanism.

Keywords: Anthocyanin; Dyslipidemia; High Fat Diet; Lipid Profile; Lactic Acid Bacteria; Synbiotic.

Dyslipidemia has a high prevalence worldwide. This condition happened as a result of unhealthy diet and lifestyle. High calories and high fat intake along with lack of routine physical activities will leads to obesity.¹ Complication of obesity with other conditions

such as hypercholesterolemia, hyperglyceridemia, hypertension, and disruption of glucose metabolism are considered as the risk factors of the rising LDL oxidation level. Furthermore, this condition will also contribute in increasing the risk of atherosclerosis plaque formation by which leads to

development of coronary heart disease and stroke.²

Lipid from food will be broken down into cholesterols, triglycerides, phospholipids, and free fatty acids during the digestion process in small intestines with triglyceride as the major form of digested fats. The absorption process of lipid upon digestion occur in the mucosal enterocytes particularly in the proximal part.³ One of dyslipidemia prevention strategy that can be done by inhibiting lipid absorption using probiotic and prebiotic lining the small intestine, which are closely related to the function of gut microbiome in the body.

There are many factors that could influence gut microbiome in the human body, including dietary, micronutrient intake, genetic, specific region of country where a person lives and antibiotic consumption.⁴ The amount of bacteria in human intestine is also closely related with interaction between host, environment, and antigen inside the human body which represent intestines protection with wide area (250-400 m²).⁵ With these factors, the modification of gut microbiome for health benefit is highly possible and one of the potential method is by the consumption of functional foods such as yogurt.

Recently, there are a lot of modification on the yogurt production process available. The source of probiotic and prebiotic can be from natural ingredients based processed food in the form of yogurt.⁶ Sweet potato (*Ipomoea batatas L.*) can be used as the prebiotic element in yogurt production. There are several types of sweet potato available which can be differentiated based on its color due to antioxidant content. The purple color in purple sweet potato represents the high content of anthocyanin.⁷

The presence of anthocyanin in purple sweet potato as natural antioxidant is quite intriguing to be explored more due to its abundance of benefits. In vitro studies had been done to discover the effect of anthocyanin extract from purple sweet potato. Several studies revealed antioxidant effect of anthocyanin through several physiological mechanism including (1) free radical-scavenging activity, (2) increasing radical oxygen absorbance capacity, (3) inhibition of Angiotensin-I Converting Enzyme, (4) inhibition of α -glucosidase, and (5) anti-mutagenic activity.⁷⁻⁹ Thus, it is fascinating to do further study on anthocyanin due to its great

potential in preventing numerous diseases related to unhealthy lifestyle along with its complication condition such as dyslipidemia, obesity, diabetes mellitus (DM) type 2, and other metabolic diseases.

Several approaches such as diet program, physical activity, and medication has been done to overcome dyslipidemia condition. However, many patients with this condition could not keep their persistency in continuing the treatment program. Therefore, another approach such as utilizing functional food to help in dyslipidemia treatment is needed. Functional food is a food product that has an improved nutritional values beyond its traditional nutrients, sensory profile, physiochemical and rheological characteristics as well as to provide therapeutic properties. Yogurt is one of functional food with combination of probiotic fermentation and natural ingredient (prebiotic) which is called synbiotic. Probiotic that commonly used in yogurt production is lactic acid bacteria (LAB), in which known to have positive effect in preventing hypercholesterolemia and obesity.^{10,11}

There are many modifications in yogurt production process. The high content of antioxidant particularly anthocyanin make it's quite intriguing to explore more on the effect of purple sweet potato as prebiotic element of yogurt. With the fortification of purple sweet potato into the yogurt, it is expected to show more positive effect on treating dyslipidemia. Thus, this experimental study aims to identify the effect of purple sweet potato yogurt (PSPY) consumption towards visceral fat weight and lipid profile on mice with high fat diet condition.

MATERIAL AND METHODS

Purple Sweet Potato Yogurt (PSPY) Making Process

Lactic acid bacteria (LAB) strains used in development of starter PSPY including: *Lactobacillus bulgaricus* ATCC 11842, *Lactobacillus acidophilus* ATCC 4356, and *Bifidobacterium longum*. These probiotics were provided and confirmed to be used in these study of PSPY by Advanced Biomedical Laboratory, Division of Microbiology and Parasitology Laboratory, Faculty of Medicine, Universitas Padjadjaran, Indonesia. Two streaks of inoculating

loop of each bacteria strain was inoculated into 100 mL of commercial low fat pasteurized milk from PT UltraJaya Milk Industry, and then incubated in 5% CO₂ incubator at 37°C for 24 hours. After incubation, starter culture from all bacteria strains was combined along with milk (10% v/v) and incubated at 37°C for 24 hours. The fermented product became mother culture of purple sweet potato yogurt (PSPY).

Purple sweet potatoes were obtained from agricultural area in Tanjung Sari, Sumedang, West Java, Indonesia. Five grams of purple sweet potato was washed and then steamed until tender. Following that, 3 grams of steamed purple sweet potato was peeled and cut into small pieces before it was mashed into puree using blender. During the blending process, warm water was added into the purple sweet potato with ratio 1:1. After that, Purple sweet potato puree was filtered using cotton fabric until purplish liquid was separated from the puree. The filtrate was then combined with low fat pasteurize milk (60% v/v) and 10% v/v of starter culture of combined bacteria. Lastly, the fortified milk was incubated at 37°C for 24 hours until it was fermented into yogurt product (Figure 1).

Animal Preparation

This experimental study used male mice (*Mus musculus*) Balb/c from Laboratory of PT. Bio Farma, West Java, Indonesia. The mice were acclimated in a cage size 41,5 x 29,5 x 20 cm with all its condition during night and day as well as its food and drink have been approved by Research Ethical Committee of Universitas Padjadjaran No. 234/UN6.KEP/EC/2019. Adaptation of the animal by providing standardized diet was done for 7 days before intervention experiment.

Purple Sweet Potato Yogurt (PSPY) Intervention

Total of 30 male mice (4-6 months old) with each weight around 20-35 gram were divided into 6 groups with different treatment on its diet: [1] standard diet (SD), [2] high fat diet (HFD), [3] HFD with 0.3 mL of PSPY [4] HFD with 0.5 mL PSPY, [5] HFD with 1 mL PSPY, [6] HFD with simvastatin. In the first 3 weeks, group 2 until 6 were given HFD treatment to induce dyslipidemia condition. Subsequently, each group was treated by respective arranged diet in the next 9 weeks. The standard diet and high fat diet used in this experimental study was obtained from Animal Model Development Division of Pharmacy Study

Program, Institut Teknologi Bandung (ITB), Indonesia. The formula was modified from previous research¹² with detail composition as shown in table 1.

Body Weight Measurement

During the experiment, body weight of the mice was measured every week using digital precision balance, brand ACS, AD-300i. The result was recorded in gram unit.

Evaluation of Cholesterol, Triglyceride, and LDL Level

Blood were harvested from retro-orbital venous sinus for investigation of total cholesterol level, triglycerides level, and LDL level. The experiment was done by using enzymatic colorimetric assay.

Cholesterol level was determined by using Cholesterol Kit Assay (LABKIT, LKBSDDT48, Chemelex SA, Barcelona). Total of 10 μ L of plasma was mixed with reagent (PIPES pH 6.9, phenol, cholesterol esterase, cholesterol oxidase, 4-aminoantipyrine) and incubated at room temperature for 10 minutes. The result was read by using spectrophotometer at wavelength 500-550 nm for 60 minutes.

Triglycerides were examined by using TG Kit (DUMOLAB, D95380). Total of 10 μ L of plasma was mixed with reagent (Good's buffer pH 7.2, 4-chlorophenol, Mg²⁺, ATP, glycerol kinase, peroxidase, lipoprotein lipase, 7-aminoantipyrine, glycerol 3-phosphate-oxidase) and incubated at 37°C for 10 minutes. The result was read using spectrophotometer at wavelength 500-546 nm for 60 minutes.

LDL level was examined using LDL Cholesterol Kit Assay (LABKIT, LKDSDDT51, Chemelex SA, Barcelona). Total of 4 μ L plasma was mixed with reagent 1 (PIPES pH 7.0, cholesterol esterase, cholesterol oxidase, catalase, N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline) and incubated at 37°C. After 5 minutes of incubation, 100 μ L of reagen 2 (PIPES pH 7.0, 4-Aminoantipyrine, peroxidase) was added into the mixture and incubation was continued for another 5 minutes. Result was read using spectrophotometer at wavelength 600nm.

Measurement of Visceral fat and Liver Weight

In the end of the experiment, visceral fats and liver were harvested to undergo weight measurement. Visceral fats were harvested from

abdominal and epididymis area. The weight measurement was performed by using digital precision balance brand ACS, AD-300i.

Intestinal Lactic Acid Bacteria Identification and Measurement

Proximal part of jejunum was harvested from mice of each group. Around 4-6 cm of jejunum was cut and put into physiological NaCl 0.9% for identification of lactic acid bacteria characteristic in mice's intestine. After that, the sample was chopped into little pieces and put into sterile vial filled with deMan, Rogosa, Sharpe (MRS) broth (OXOID, CM0359). Then the sample was stored at 37°C for 24 hours. Following that, total plate count (TPC) was done on bacteria culture from the sample. Dilution of 1 mL sample was done 6 times (10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶). Each dilution was then cultured on MRS agar (OXOID, CM0361) and incubated for 24-48 hours. After that, colony number (CFU/mL) of each sample was calculated.

The presence of lactic acid bacteria was identified by using catalase test. Bacteria was taken using inoculation loop and then mixed into 3 mL

of physiological NaCl 0.9%. After that, one drop of H₂O₂ was put on the sample. The formation of bubble on the sample upon addition of H₂O₂ will determines the presence of bacteria other than lactic acid bacteria.¹³

Gram staining (ST-REAGENSIA, 055-0746, Indonesia) was done on bacteria culture for morphological identification of the intestinal bacteria of mice models. Bacterial colony was taken using inoculation loop and mixed with 1 drop of physiological NaCl 0.9% on object glass and then let it air dry followed by gram staining with sequence: Crystal violet as primary stain, lugol, alcohol, and lastly safranin as counter stain. Morphology of the stained bacteria was observed using microscope Olympus CX23LEDRF51 with serial number 5M8758120152.

Statistical Analysis

Presented data is Standard Error Mean (SEM). Results were analyzed using one-way ANOVA and post hoc tukey multiple comparison test using GraphPad-Prism 7. p values < 0.05 considered significant.

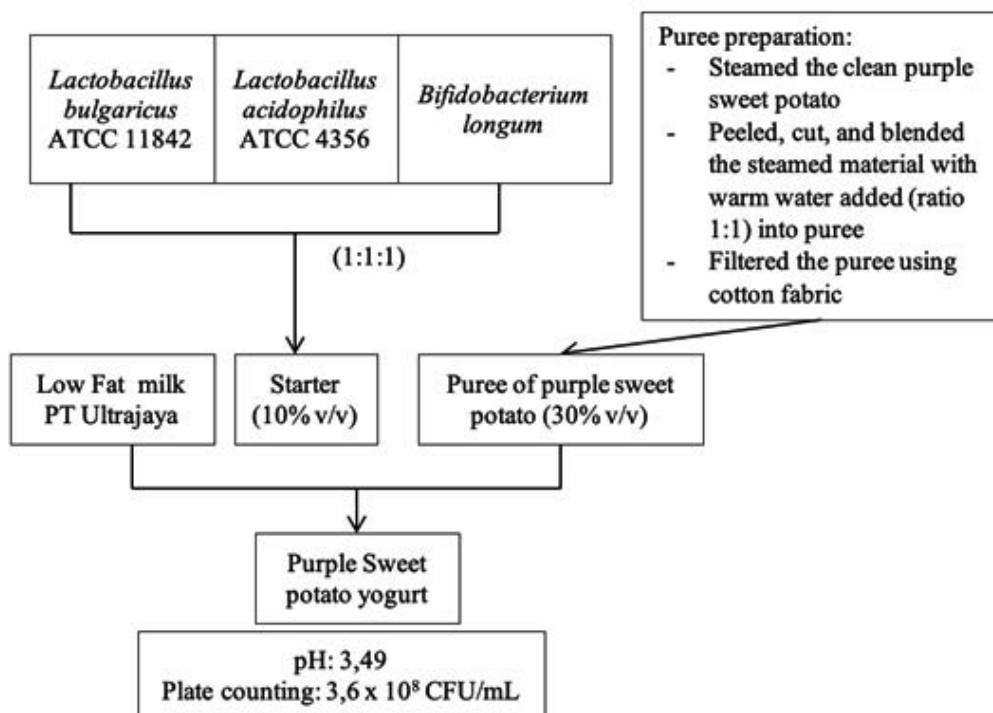


Fig. 1. Purple sweet potato yogurt production flowchart

RESULTS

Intestinal Lactic Acid Bacteria Colony Profile

After purple sweet potato yogurt (PSPY) intervention, microbiota from intestine were cultured on MRS agar to identify and calculate the amount of lactic acid bacteria. The result revealed that total amount of lactic acid bacteria colony from HFD group relatively lower compare to SD group. From the figure 2A, in can be determined that administration of 0.3 mL of PSPY could significantly increase the growth of lactic acid

bacteria in the intestine. In addition, catalase test and gram staining experiment of the bacterial culture showed bacillus form of gram negative bacteria (Figure 2B and 2C).

Purple Sweet Potato Yogurt (PSPY) Effect on Inhibiting the Weight Gain

Elevation of body weight on mice group with High Fat Diet significantly higher compared to mice group with standard diet. High content of fats and calories from the diet contributed in body weight elevation as it increased fat deposit. This study showed that administration of PSPY on high

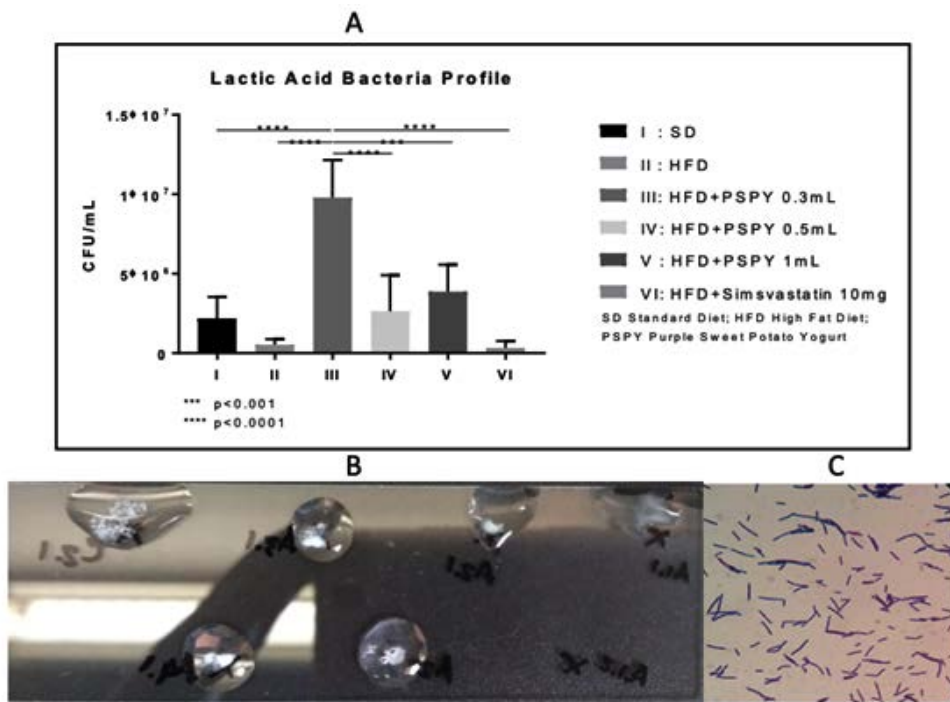


Fig. 2. (A)Average ($\pm SEM$) of lactic acid bacteria colonies from mice’s intestine upon purple sweet potato yogurt intervention. *** represent $p<0.001$, **** represent $p<0.0001$ (B) Catalase test result. (C) Gram staining result of lactic acid bacteria from mice’s intestine

Table 1. Standard Diet (SD) and High Fat Diet (HFD) Composition

No.	Analysis Parameters	Analysis result		Result Unit	Testing Method
		SD	HFD		
1.	Moisture	9.23	4.74	%	SNI-01-2891-1992 <i>By Difference</i> -
2.	Ash content	16.53	9.19	%	
3.	Protein content	13.63	13.96	%	
4.	Fat content	6.84	35.25	%	
5.	Carbohydrate content	53.77	36.88	%	
6.	Total Calories	331.16	520.61	Kkal	

fat diet mice group could inhibit the elevation of body weight (Figure 3.).

Purple Sweet Potato Yogurt (PSPY) Effect on Improvement of Lipid Profiles

Group with high fat diet intake demonstrate the elevation of circulating lipid profile including cholesterol, triglycerides, and LDL level compared to group which took standard diet. However, consumption of PSPY by group which took HFD showed the improvement of its lipid profiles. The 3rd group showed the most favorable result in lipid profile improvement even compare to positive control group (treated with simvastatin). The result also showed indication of increasing of lipid profiles along with the increase of the dosage of yogurt administered. Nevertheless, the improvement of lipid profile in the group treated by in general can be seen (Figure 4.).

Purple Sweet Potato Yogurt (PSPY) Effects on Visceral Fats and Liver Weight

The weight of visceral fats and liver of HFD group were significantly higher compared to the SD group. The 3rd experiment group showed the most favorable outcome in term of reducing liver and visceral fat weight even compared to control positive group treated by simvastatin. However, this experiment also revealed the increasing of visceral fat and liver organ following the increase dose of yogurt treated to group 4 and 5 (Figure 5).

DISCUSSION

Probiotic used in fermentation process had been reported to contribute in formation of nutritional component that is beneficial for health, such as protein, vitamin, and antioxidant.¹⁴ In addition, fortification of yogurt using various natural ingredients had been done a lot.¹⁵⁻¹⁸ This kind of fortification was expected to help improve the health benefit of yogurt for its consumer. Nutrition fact screening has been done on purple sweet potato yogurt (PSPY) used in this experimental study. The result of the screening revealed some amount of anthocyanin content, a unique characteristic of purple sweet potato, which was a type of flavonoid that had antioxidant activity.^{8,15,18} The amount of anthocyanin content in the PSPY stated in nutritional fact screening was 143.06mg/dL. Further, acidity level as well as total colony of bacteria in the yogurt product had been evaluated and both results were within normal range.

The finding of this study showed significant result on the improvement on lipid profile including total cholesterol, triglycerides, and LDL level ($p < 0.05$) due to anthocyanin composition in the purple sweet potato yogurt. Previous studies reported the effect of anthocyanin from fruits in lowering de novo fatty acids synthesis, which is suspected due to the activation of AMP-activated protein kinase (AMPK).^{18,19}

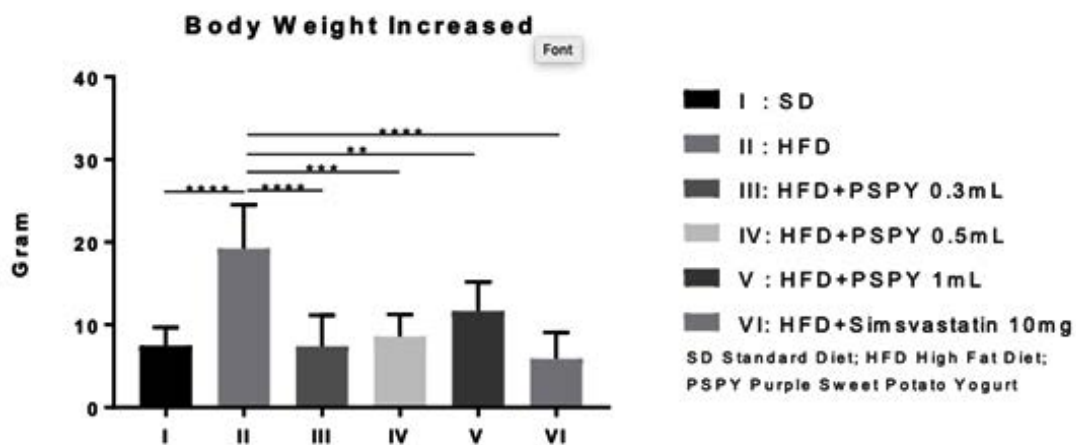


Fig. 3. Average ($\pm SEM$) increase of body weight. ** represent $p < 0.01$, *** represent $p < 0.001$, **** represent $p < 0.0001$

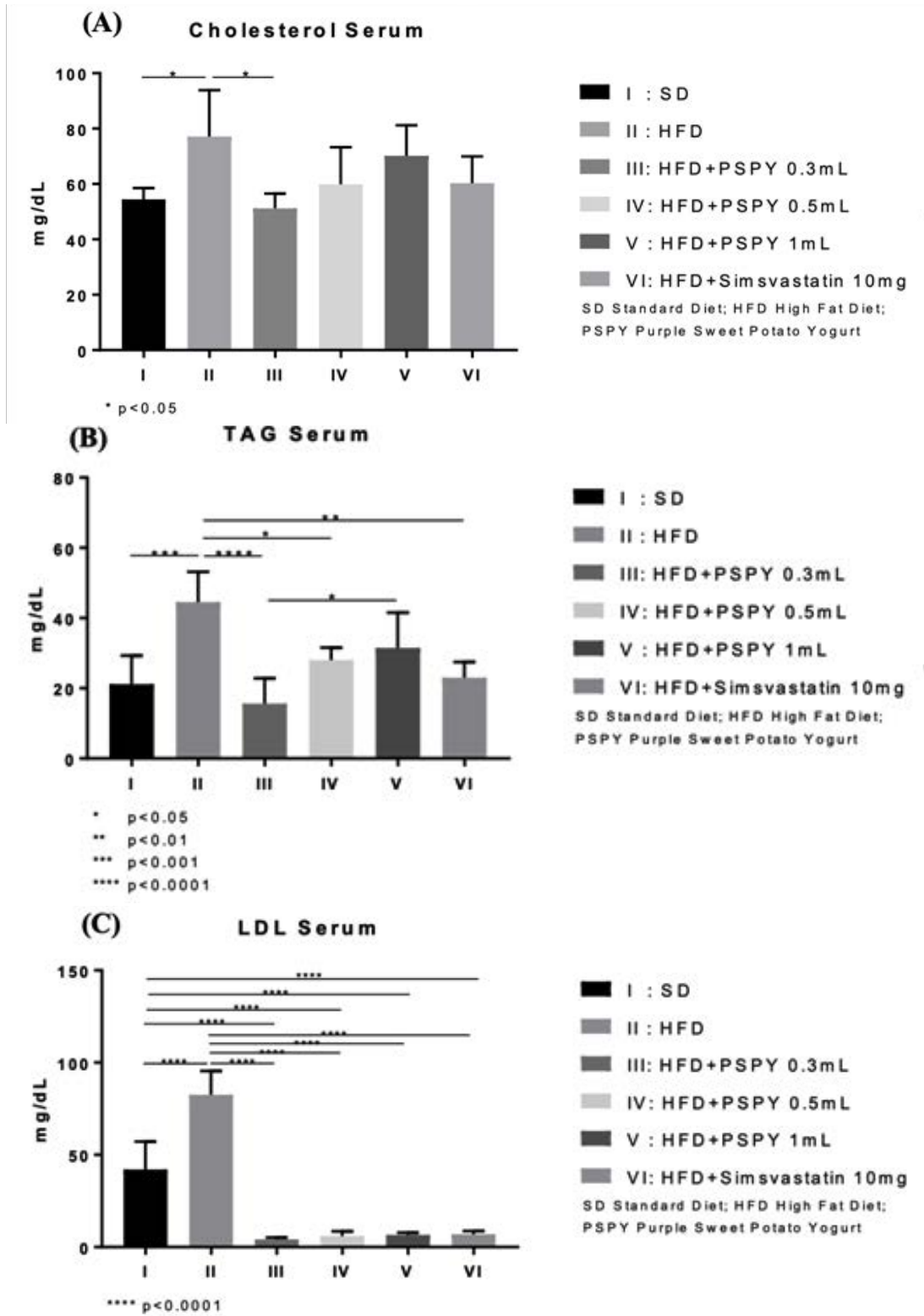


Fig. 4. (A) Cholesterol, (B) triglycerides, and (C) LDL levels of each experimental group. * represent $p<0.05$, ** represent $p<0.01$, *** represent $p<0.001$, **** represent $p<0.0001$

The activation of AMPK caused the inactivation of malonyl CoA decarboxylase (MCD), which led to the decreased of malonyl CoA level. Malonyl CoA itself was essential in de novo synthesis of fatty acids. Thus, de novo fatty acid synthesis in the liver was reduced due to the low level of malonyl CoA.²⁰ Therefore, the activation of AMPK led to the inhibition of fatty acid synthesis as well as the oxidation of fatty acid. Thus, it was suggested that the anthocyanin from purple sweet potato could reduce de novo synthesis of fatty acid.

This study revealed that high fat diet group had significantly lower total lactic acid bacteria colony compared to other experimental groups. This finding was consistent with previous study by Turnbaugh and his colleagues, in which they

also found lowering amount of 50% of Firmicutes microorganism such as *Lactobacillus* in obesity related diet study.¹⁹ Another study also reported that high fat diet intake decreased the composition of Bacteroidetes colony compared to low fat diet group.²⁰

This study also found the decreasing of lactic acid bacteria colony in the mice intestine of the group which consumed higher dosage of PSPY. The possible reason for this finding was because in higher dose group, the bacteria created engagement with villi of small intestine where it propagated further until it exceeded its stationer phase. In this condition, the growth rate of the bacteria would be equal with its death rate, which means the colony would enter the death phase. Thus, the result of lower bacteria colony in higher dose group occur.²¹

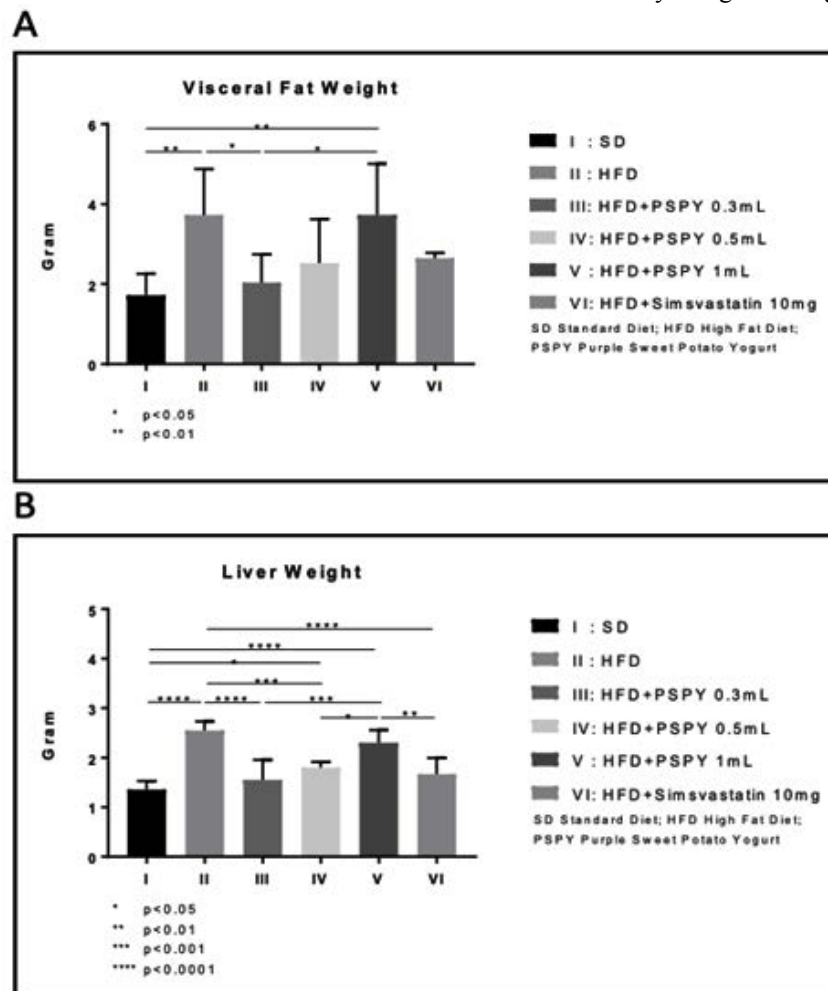


Fig. 5. Comparison of visceral fat weight and liver fat weight between experimental groups. * represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$, **** represents $p < 0.0001$

Consumption of fermented milk would alter the gut microflora. The consumption of probiotic fermented milk and yogurt could increase colony amount of Bifidobacterium in adult human.²² Colony number of lactic acid bacteria was significantly high in the group that consume PSPY with dosage 0.3 mL/20 mg of mice's body weight compared to other groups. This discovery showed that purple sweet potato could promote proliferation of lactic acid bacteria in high fat diet group study.

Probiotic which entered digestive system would be exposed to different type of environment including the acidity level of the environment. Therefore, only bacteria which could survived the harsh condition could reach and colonized in the intestine and colon.²³ The isolation of microflora from human intestine had been done numerously and the most common type of bacteria identified was lactic acid bacteria. It is hypothesized that lactic acid bacteria had the tendency to be able to reach small intestine after underwent harsh environment condition changes in gastrointestinal track.²⁴ Lactic acid bacteria harvested from mice small intestine was identified to be classified as bacillus gram positive bacteria with negative result on catalase test. These results was corresponded with the characteristic of lactic acid bacteria isolated from milk product.²⁵ This study also demonstrated that effect of PSPY product in inhibiting weight gain, and decreasing visceral fat and liver weight in high fat diet mice model.

CONCLUSION

The best dosage of purple sweet potato yogurt to improve health condition of high fat diet mice model was 0.3 mL/20 gram of mice weight. The group treated with this dosage showed favorable outcome in term of total colony number of lactic acid bacteria, improvement of lipid profiles including total cholesterol, triglycerides and LDL level, with better visceral fat and liver weight. However, the product did not reveal anti-obesity effect due to insignificant body weight changed of the experimental model. Further study is needed to explore more detail on its molecular mechanism and also the toxicity dosage of the purple sweet potato yogurt product.

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