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International Conference on Food and Bio-Industry 2019 IOP Conf. Series: Earth and Environmental Science 443 (2020) 012106 IOP Publishing doi:10.1088/1755-1315/443/1/012106 1 The influence of black rice bran aqoueus extract on blood and spleen profiles E P Nurlaili1, R A S Lestari2 and S Hartati3 1 Faculty of Agricultural Technology, 17 Agustus 1945 University (UNTAG), Semarang, Indonesia 2 Faculty of Engineering, 17 Agustus 1945 University (UNTAG), Semarang, Indonesia.

3 Faculty of Agricultural, Bangun Nusantara University (UNIVET), Sukoharjo, Indonesia Email: enny.purwati@gmail.com Abstract. Consumption of foods high in fat and sugar triggers dyslipidemia which can cause atherosclerosis and heart disease, inflammation. Dyslipidemia can be prevented by antioxidants, including those contained in pigmented rice, namely black rice, especially in bran.

This study aimed to evaluate the effect of soygurt enriched with black rice bran extract on the blood and spleen profile of white rats (Rattus norvegicus). The results of the observations on the blood profile showed that the treatment of black rice bran extract showed improvement in existing blood cells compared to control and placebo treatments, as seen in the shape, size, and color of the profile.

The results of the histological analysis on spleen organs showed very significant results, in treatment with black rice bran extract showed only a little damage to the spleen organ compared to other treatments. Sensory test results showed the color and flavor test of the respondent liked, while the taste showed liked very much. 1.

Introduction The people's lifestyle in modern times tends to be instant. The technological developments, as well as consumptive behavioral patterns, trigger poor patterns of life, especially in food consumption which can cause health problems. The decline in the quality of public health is partly due to the imbalance of eating and physical activities.

People who tend to high-sugar and fat diets without adequate physical activities can trigger dyslipidemia which is at high risk of cardiovascular disease [1]. These diseases include atherosclerosis, hypertension, stroke and coronary heart disease [2]. Dyslipidemia is caused by excess calorie intake, especially fat, which if not used for activities can cause an increase in blood fat levels.

High-fat diets, especially cholesterol, are a major cause of dyslipidemia [3]. This condition can cause an increase in LDL levels in the blood. LDL oxidation triggers the formation of plaques (clots in blood vessels) which are the main causes of atherosclerosis, coronary heart disease and stroke [4]. The condition of dyslipidemia has many negative impacts so that a healthy and balanced diet is needed.

Inflammation is the reaction of living tissue to all forms of the lesion in the form of a vascular reaction which its results are the delivery of fluids, dissolved substances and cells from the blood circulation to the interstitial tissue in the area of injury or necrosis [5]. Inflammation aims to repair damaged tissue International Conference on Food and Bio-Industry 2019 IOP Conf.

Series: Earth and Environmental Science 443 (2020) 012106 IOP Publishing doi:10.1088/1755-1315/443/1/012106 2 and defend against infection [6]. Signs of inflammation are redness (rubor), heat, pain (dolor), swelling (tumor) [6] and function less [7]. One effective way to overcome dyslipidemia and inflammation is to consume foods that contain lots of antioxidants.

Antioxidants have the properties to neutralize disease-causing free radicals caused by dyslipidemia conditions such as atherosclerosis [8]. Bran is a brown layer located between rice seeds and rice outer shell, which is rich in protein, oil, and other nutrients. This bran is an abundant rice production waste in Indonesia. So that this bran can potentially be used for various products that have high added value.

One type of rice found in Indonesia is black rice. Like other rice, the black rice consists of rice hull, rice, and bran. Bran of black rice has high antioxidant content, especially anthocyanin. The anthocyanin content in Thai black rice bran is 11.27 ± 0.38 E/g [9]. The use of bran as an anthocyanin sourced food is constrained by the presence of absorption inhibitors, including phytic acid so that the component needs to be reduced by extraction. The extract obtained was used to apply it to the test in vivo.

Giving a diet of red and black rice can increase blood antioxidant levels and reduce atherosclerotic plaque formation in dyslipidemia rabbits [10]. Antioxidants are known to help improving atherosclerosis in the aorta. Antioxidant-rich extracts obtained from black rice can increase the stability of plaque in rats that have a deficiency of Apolipoprotein-E.

This mechanism is related to the antioxidant ability to reduce fat and as an anti-inflammatory. Antioxidant-rich extracts are thought to have a therapeutic effect on patients with atherosclerotic plaques and prevent sub-thrombus [11]. A study in Brazil also showed that giving a diet containing cultivar black rice IAC600 can control the condition of dyslipidemia in white rats [12].

Soygurt (soy milk yogurt) is a fermented soy milk food product containing off-flavor (beany flavor) that is less preferred. The cultures commonly used in making soygurt are probiotic bacteria that are Lactobacillus bulgaricus and Streptococcus thermophilus. Soygurt can provide many health benefits including preventing degenerative diseases such as cancer, osteoporosis, and coronary heart disease.

Besides that, it also helps prevent diarrhea and increase cholesterol. Although it has better benefits than white rice bran, black rice bran has not been widely used as a source of antioxidants, especially anthocyanins. Based on the description above, it is necessary to conduct this research to study the effects of antidyslipidemia soygurt enriched with anthocyanin of black rice bran extract in white rats, among others, in terms of its histological. 2. Research method 2.1.

Main material The material used is Cibeusi variety of black rice from Subang. The samples obtained were then milled and rinsed with a rinsing machine for 5 seconds to obtain bran. The bran is stored in the freezer (-20 °C) until used. Other materials are soybeans purchased at the Semarang market. 2.2.

Chemicals and research tools Chemilused ncl , etiacihexaner d -93G and AIN-93M (American Institute of Nutrition) [13], deionized water, obtained from Sigma and Merck with pro analysis specifications (pa) Materials for histological analysis include ethanol, hematoxylin and eosin dyes. For this study several types of equipment will be used, among others, glassware, analytical scales, ovens, water bath shakers, muffle furnaces, UV-VIS spectrometers, vortex, centrifuges at 4 ° C, rotary evaporator, freeze dryer. 2.3. Experimental animals The experimental animals used are 3 weeks aged-male Wistar rats. 2.4.

Rats feed Tat feed was AIN -93G and AIN-93M. International Conference on Food and Bio-Industry 2019 IOP Conf. Series: Earth and Environmental Science 443 (2020) 012106 IOP Publishing doi:10.1088/1755-1315/443/1/012106 3 2.5. Research stages 2.5.1. Preparing the experimental animals.

The experimental animals used were 24 male Wistar white rats, 3 weeks old, which were obtained from LPPT (Integrated Research Development Institute) of Gadjah Mada University. Determination of the number of experimental animals refers to the Federer formula [14], (n-1) (k- Before the treatment, the experimental animal previously undergoes an adaptation period for 7 days and are fed with ad libitum. The remaining feed is weighed every day. After the adaptation period, the animal tries to weigh it.

2.5.2. Preparation of black rice bran extract. Preparation of black rice bran extract using black rice bran extracted with a solution of 3% acetic acid in demineralized water (ratio 1:10) and put in a shaker at 40 °C, speed 125 rpm, for 60 minutes then stored at 4 °C for 24 hours.

Then filtering was carried out, thickened with a rotary evaporator for 4 hours at 40 °C and stored in a freeze dryer (-20 °C) until used. 2.5.3. Making soygurt. Making soygurt is carried out according to [14]. 1000 ml of soy milk is prepared and then 5% of sugar is added. Then pasteurized at 80 90 °C for 30 minutes. Then cooled to reach 40 ° C and inoculated with starter cultures of Streptococcus thermophillus and Lactobacillus bulgaricus each of 2.5% of the volume of soy milk.

Then incubated for 15 hours at room temperature. After that, it is cooled to 4 ° C. 2.5.4. Making feed. Making feed for experimental animals, according to Reeves et al 1993, namely AIN-93G and AIN-93M. 2.5.5. Maintenance, euthanization, and surgery of experimental animals.

Before entering the trial period, all rats had undergone an adaptation period of 7 days, by giving a standard diet (AIN-93G) and deionized water drinks in ad libitum. Furthermore, all rats are conditioned to be dyslipidemia by being fed AIN-93G and induced with fructose and cholesterol (depletion period). The rats that had experienced dyslipidemia were then divided into 4 groups @ 6 animals and were kept for 5 weeks in individual cages, with the provision of basal diets and deionized water drinks in ad libitum (repository period).

During the repetition period, the rats have been fed with soygurt drink that has been enriched with anthocyanin black rice bran extract in force-feeding. From the 24 rats, they are divided into 4 groups, each consisting of 6 rats, namely: Group 1 was fed with

AIN-93M standard food and deionized water (as placebo group = P); Group 2 was fed with AIN-93M standard food and soygurt (as control group = K); Group 3 was fed with AIN-93M standard food and 50 mg = E-50 anthocyanin enriched soygurt; Group 4 was fed with AIN-93M standard food and anthocyanin enriched soygurt 100 mg = E-100).

Blood sampling is carried out through retroorbital flexus, and then the surgeon is conducted and the spleen is taken. At the end of the research, an analysis of blood and spleen histology was carried out. 2.5.6. Data analysis. Data analysis is performed by analysis of one-way variants (One Way Anova) with Minitab 17 at a significance level of 5%.

If there are significant differences, it is proceeding with the Tukey's test. 3. Results and discussions 3.1. Chemical test results Black rice is very rare because aleurone and endosperm produce high-intensity anthocyanins so that it is thick purple or almost black. Anthocyanin is one of the main groups of pigments in plants [16], in all high-level parts of the plant [17], in plants that flower and produce colors from dark red to blue on flowers, fruits, and leaves [16].

All anthocyanins are derivatives of flavillium cations (3, 5, 7, 4 'tetrahydroxiflavilium) which are the basic structures of anthocyanidin [18]. International Conference on Food and Bio-Industry 2019 IOP Conf. Series: Earth and Environmental Science 443 (2020) 012106 IOP Publishing doi:10.1088/1755-1315/443/1/012106 4 Observation of anthocyanin levels will prove that there is an anthocyanin pigment content indicating an indication of glycosidic bonds, which is one of the characters of anthocyanin pigment which consists of aglycone (as anthocyanidin) and glycone as the sugar compound it binds [19].

The two varieties have relatively more equal total sugar content, which is 8.33 – 8.33%, which is relatively high compared to the total sugar content in kana flowers of 3.2% and water henna plant of only 2.75% [20]. The proximate analysis of black rice performed showed a protein content of 9.03%; 1.66% fat; 0.56% ash; water 14.55% and carbohydrate levels (by difference) of 74.20%.

The anthocyanin level in black rice is 0.37 mg/g, whereas in the liquid extract is 0.0035 mg/g and the dry extract is 4.22 mg/g. As for the results of the physical properties of black rice with color testing with a chromameter tool, it shows that the L * value of 36.06 describes a color that tends to be blackish, a value of 5.10 indicates a reddish color and a b value of 2.29 indicating colors tend to contain green.

Soybean is a shrub that grows upright with a height reaching 50 cm, the seeds are pod-shaped and belong to the family of Leguminosae (beans). Soybeans are a source of protein and vegetable fats which are very important in life. In more detail the chemical analysis of raw materials for soybeans, soy milk and soybean varieties (soygurt) purchased at the Semarang market is a proximate analysis consisting of analysis of protein, fat, ash, water and carbohydrate levels. The average value of the proximate analysis results can be seen in table 1. Table 1.

Results of proximate analysis of soybeans, soy milk and soygurt. No Chemical Analysis Soybeans (%) Soy milk (%) Soygurt (%) 1 Protein content 28.58 1.72 4.12 2 Fat content 17.94 1.56 1.49 3 Ash content 4.88 0.31 0.81 4 Water content 11.10 93.68 79.31 5 Carbohydrate content * 37.50 2.73 14.27 *By difference Based on the proximate analysis performed, the levels of protein, fat, ash, water and carbohydrate content contained in local varieties of soybeans were 28.58% respectively; 17.94%; 4.88%; 11.10% and 37.50%.

Observations on protein, fat, ash, water and carbohydrate levels contained in soy milk prove that there are lower protein levels than soybean raw materials. While the results of the proximate analysis on soybean fermented products (soygurt) respectively for protein content of 4.12%, fat 1.49%, ash 0.81%, water 79.31% and carbohydrate content 14.27%. Soybeans contain 35% protein while protein levels in superior varieties can reach 40 – 43%.

From the results of the research, it was found that protein levels were lower than these results. 3.2. Sensory test results Sensory tests carried out in this research used a hedonic test (preference test) [21], using 5 scales namely 1 = very dislike; 2 = dislike; 3 = Neutral; 4 = likes and 5 = very like.

The hedonic test aims to determine the level of preference for the color, flavor, and taste of soygurt enriched with black rice bran extract and controls. Test results for the color, flavor, and taste of soygurt are listed in figure 1. International Conference on Food and Bio-Industry 2019 IOP Conf. Series: Earth and Environmental Science 443 (2020) 012106 IOP Publishing doi:10.1088/1755-1315/443/1/012106 5 Figure 1. Average Numerical scale of panelists' likes.

The data in figure 1 shows that the color, flavor, and taste of soygurt enriched with black rice bran extract and controls in the range of likes to very like. The level of preference of the panelists for the three types of soygurt is illustrated in the spider web in figure 2, the results show that the color, flavor, and taste of soygurt enriched with E-100 black rice bran extract are in the outer circle followed by soygurt enriched with extract E-50 black rice bran and controls.

This indicates that the color, flavor, and taste of soygurt enriched with E-100 black rice bran extract is preferred compared to soygurt enriched with E-50 black rice bran extract and Control. There is a strong suspicion that the panelist habit factor in consuming soygurt causes the E-100 treatment to be preferred. Statistical analysis to determine the panelists' response to the level of soygurt preference was done through a parametric approach through Complete Random Design (CRD). Parametric statistical analysis through a variance analysis approach is by transforming numerical scale data with square roots to approach a normal distribution.

According to [22] stated that the distribution of data that is doubtful in normality must be transformed to approach a normal distribution. Statistical analysis of the degree of preference of soygurts from various treatments through this parametric approach showed results that were not significantly different in all tests. Figure 2. Spider web hedonic test. 3.3.

Blood profile According to research that has been done, anthocyanin pigments and other flavonoids have been shown to have positive effects on health [18]. To find out the blood profile in this research, it can be seen in figure 3 below. 3 . 3 3 3 . 4 0 3 . 5 3 3 . 7 3 4 . 0 0 4 . 1 3 4 . 1 3 4 . 0 7 4 . 5 3 0.00 1.00 2.00 3.00 4.00 5.00 COLOUR FLAVOUR TASTE Numeric Scale Hedonic Test C E -50 E -100 0.00 1.00 2.00 3.00 4.00 5.00 COLOUR FLAVOUR TASTE C E -50 E -100 International Conference on Food and Bio-Industry 2019 IOP Conf.

Series: Earth and Environmental Science 443 (2020) 012106 IOP Publishing doi:10.1088/1755-1315/443/1/012106 6 (a) (b) (c) (d) Figure 3. Blood profile: (a) Group P (AIN-93M standard feed diet + demineralized water); (b) Group K (standard AIN-93M + soygurt feed); (c) E-50 group (standard AIN-93M feed + black rice bran extract 50 mg); (d) E-100 group (standard AIN-93M feed + black rice bran extract 100 mg).

Remarks: The group of rats received a standard AIN-93M + demineralized water feed diet (P = placebo); standard AIN-93M + soygurt feed diet (K = control), AIN-93M standard feed + black rice bran extract 50 mg (E-50), and AIN-93M standard feed + black rice bran extract 100 mg (E-100). The results of observations on blood profiles in this study showed that the E-50 and E-100 treatments appeared to have improved blood cells compared to control and placebo treatments, it can be seen from the shape, size, and color of the profile. This turns out that anthocyanin can improve the blood profile of dyslipidemia.

Anthocyanin can inhibit the clumping of red blood cell pieces, stimulate the production of nitric oxide which can dilate blood vessels, inhibit the growth of cancer cells. In addition to its potential as an antioxidant and free radical scavenger, anthocyanin also has several properties such as hepatoprotective, antithrombotic, anti-inflammatory and antiviral [23]. The antiradical properties of anthocyanins are mainly hydroxyl radicals, superoxide anions, peroxyl radicals, and alkoxyl [24].

According to [25] research, the anthocyanin-containing black rice bran extract can prevent hypertriglyceridemia and anemia by reducing triglyceride levels and increasing hemoglobin levels. Whereas according to stated that sweet-sour and astringent fruit skin anthocyanin can reduce low-density lipoprotein (LDL) in Wistar strain rats that have hypercholesterolemia, with the best decrease in P3 is 54.5% for the administration of 69 g / 150 BB extract.

Anthocyanin of sweet-sour and astringent fruit skin can also increase high- density lipoprotein (HDL) after administration of anthocyanin extract from sweet-sour and astringent International Conference on Food and Bio-Industry 2019 IOP Conf. Series: Earth and Environmental Science 443 (2020) 012106 IOP Publishing doi:10.1088/1755-1315/443/1/012106 7 fruit in Wistar strain rats that have hypercholesterolemia, with the best increase being shown by P3 which is 16.6% 3.4.

Spleen profile The spleen is the largest lymphoid organ in the body and the only organ involved in blood filtration so that it is an important organ in defense against antigens in the blood. A spleen is also a place for the destruction of old erythrocytes as well as a place for producing antibodies and active lymphocytes, which are delivered to the blood [26]. To find out the histopathological picture of the spleen with hematoxylin- eosin staining in this research can be seen in figure 4. (a) (b) (c) (d) Figure 4.

Histopathological picture of the spleen with hematoxylin-eosin staining: (a) Group P (AIN-93M standard feed diet + demineralized water); (b) Group K (standard AIN-93M + soygurt feed); (c) E-50 group (standard AIN-93M feed + black rice bran extract 50 mg); (d) E-100 group (standard AIN-93M feed + black rice bran extract 100 mg). Remarks: The group of rats received a standard AIN-93M + demineralized water feed diet (P = placebo); standard AIN-93M + soygurt feed diet (K = control), AIN-93M standard feed + black rice bran extract 50 mg (E-50), and AIN-93M standard feed + black rice bran extract 100 mg (E-100) The results of histopathological examination of the spleen of white rats on placebo from showing the entire spleen of all-white rats experienced a slight necrose, but also there was bleeding.

Necrosa control In the control treatment experienced a little necrosa, but also there was also moderate bleeding most and almost all the spleen. The results of observations on the spleen histopathology of white rats (Rattus norvegicus) in all treatment groups are presented in figure 4. An E-50 group is a group that by histopathological observation has a good description. Necrosis in the E-50 treatment has improved.

For International Conference on Food and Bio-Industry 2019 IOP Conf. Series: Earth and Environmental Science 443 (2020) 012106 IOP Publishing doi:10.1088/1755-1315/443/1/012106 8 the spleen, there is moderate bleeding which is less than placebo and control. The E-100 group is the group that has the best histopathological observation and bleeding is multifocal.

Bleeding in this study almost occurred in all treatment groups. According to [20] stated that blood flow through the spleen red pulp can be through closed circulation, penicillin arterioles or branched capillaries are directly related to sinusoids so that blood is always covered by the vascular endothelium.

Whereas open circulation, penicillin arterioles have an open end that allows blood into the splenic cord stroma. According to Guyton and Hall [27], stated that this condition may be caused by the spleen itself which functions to filtrate blood and is tasked with removing materials that are not needed from the blood such as damaged red blood cells, and play a role in mobilizing blood if physiological activity increases.

This bleeding can also be caused by rats that are used to experience trauma, causing bleeding in the spleen. Necrosis can be caused by several things including lack of blood supply, toxins, no innervation of nerves, temperature, active radio rays and mechanical trauma. Cholesterol is a sterol found in animal tissues both in free and bound forms.

Cholesterol is an important biomolecule as a component of cell membranes and is a precursor of steroid hormones and bile acids. Cholesterol is synthesized in the liver and small intestine. In the liver, cholesterol helps the absorption of triglycerides and fat-soluble vitamins. In blood, cholesterol is bound to lipoprotein which consists of chylomicron, VLDL, LDL, and HDL. Each lipoprotein has a function.

Chylomicron transports cholesterol that has just been formed in the intestine to be carried into the spleen. VLDL is responsible for carrying cholesterol from the liver to muscle tissue. LDL transports cholesterol in the blood plasma to exchange substances. But in carrying out its duties, LDL particles easily attach to the walls of blood vessels.

HDL is responsible for capturing cholesterol free of membrane cells that are dead and transported back to the liver. Black rice has higher anthocyanin than the white one. This shows that the antioxidant activity of the rice is also higher, this is evidenced by the decrease in aortic wall thickening after rats are given anthocyanin treatment. 4.

Conclusions Based on the results of the research that has been done, it can be concluded that: The results of profiles of soybeans, soy milk, soygurt showed that the protein content in soygurt was greater than soy milk. The color, flavor, and taste of soygurt enriched with E-100 black rice bran extract are preferred compared to soygurt enriched with E-50 black rice bran extract and control. The statistical approach with the completely randomized design of color, flavor, and taste of the three soygurt was not significantly different.

The results of the blood profile analysis showed an improvement in the existing blood cells compared to the control and placebo treatments, seen from the shape, size, and color of the profile. The results of the histological analysis on spleen organs showed very significant results, in treatment with black rice bran extract showed only a little damage to the spleen organ compared to other treatments. Fighting leaf ethanol extract at a dose of 100 mg/kg bb and 150 mg/kg BW can improve damaged spleen tissue structure.

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