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IMUNOMODULATOR TEST ON YOGHURT GREEN BEAN Sprouts ON THE IMMUNE SYSTEM

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ABSTRACT

The development of the lifestyle of modern society is required to be able to survive in the midst of activities, especially during a pandemic about the awareness of the importance of maintaining a healthy body, especially digestive health, namely by consuming yogurt. One of the probiotic products that contain lactic acid bacteria is yogurt. Yoghurt, one of the probiotic products produced through LAB fermentation, contains Lactobacillus bulgaricus and Streptococcus thermophilus. Yoghurt is reported to increase nutritional and antioxidant content, vitamins and minerals, and dietary fibre it is beneficial for health, and to improve antioxidant status and immunity.

Local food ingredients that can be used as basic ingredients in making yogurt include green bean sprouts or known as bean sprouts. Green bean sprouts or bean sprouts are food ingredients that can be used as functional food, where these foodstuffs are very easy to find and need to be diversified in order to have high economic value.

This type of research is pure experimental. The research variables are mung bean sprout yogurt and immunoglobulin. Data were expressed as mean ± standard mean (S.E.M) and statistical analysis was performed using one-way ANOVA followed by Duncan's test.

The results: The highest number of rat leukocyte cells occurred in the group of white rats given 6 ml/200 gBB of yogurt, which was 20,100 leukocyte cells. And the low is the normal group, namely 9460 leukocyte cells. This shows the activation of the body's immune system by the presence of immunomodulators in building the immune system as a result of the Hepatitis B vaccine. Giving bean sprouts yogurt can increase the HBsAb antibody titer and the highest dose of antibody titer is 6 ml/200 g body weight in white rats.

Keywords: Green Bean Sprout Yogurt, Immunomodulator, Immune system

BACKGROUND

The world is currently in the middle of a COVID-19 pandemic. At the end of 2019, CoV infections began to enter Wuhan Hubei, China. Covid 19 has emerged as a multisystem and multi-organ disorder ranging from non-specific flu-like symptoms to pneumonia and acute respiratory distress syndrome, multiple organ failure and death. (Baud et al, 2020; Chen et al, 2020, Infusini et al, 2020).

In this pandemic situation, the role of non-pharmacological substances with herbs, probiotics and prebiotics can help in increasing the immunity of a person who is experiencing an infection either due to the corona virus or other infectious diseases. Certain fermented foods, probiotics and prebiotics can produce viable microbes with gut immunity-boosting potential. (Infusini et al, 2020).

The development of the lifestyle of modern society is required to be able to survive in the midst of activities, especially during a pandemic about the awareness of the importance of maintaining a healthy body, especially digestive health, namely by consuming yogurt. Yoghurt, one of the probiotic products produced through LAB fermentation, contains Lactobacillus bulgaricus and Streptococcus thermophilus (Guo et al., 2013)

Yogurt which is popular in Indonesia is yogurt which is made from cow's milk. Many dairy products have been developed from animal sources but only a few yogurts are made from plant-based dairy products. Yogurt products from vegetable milk actually have a lot of potential to be developed because they have high nutritional content, apart from being seen from the relatively cheaper price compared to yogurt from animal milk, they are easily affordable by all people and also have nutritional content that is competitive with animal milk yogurt products.

Consumption of yogurt today is fairly good, because people in general already know that yogurt is one of the probiotic drinks that is very good for health. The production of yogurt is now diversified, not only using dairy products but also using local plant-based food ingredients with various flavors. Local food ingredients that can be used as basic ingredients in making yogurt include green bean sprouts or known as bean sprouts. Green bean sprouts or bean sprouts are food ingredients that can be used as functional food, where these foodstuffs are very easy to find and need to be diversified in order to have high economic value.

One type of nut are rich in antioxidant is mung beans (Vigna Radiata). Besides, mung beans are also high in protein, amino acids, oligosaccharides, and polyphenols. Polyphenol compounds are known to be the main contributors as antioxidants, anti-inflammatory, and anti-tumour. However, high oligosaccharides in nuts can cause flatulence, it is important to do processing with soaking and germination. According to Winarsi et al. (2019), the phenolic content of mung bean-based yoghurt that has been soaked for 12 hrs reaches 525.958±48.9 mg GAE/L, Germination can eliminate the many flavours in the red bean sprouts yoghurt, and even increase its phenolic content. Thus, germination provides several benefits, such as reducing the anti-nutritional content, increasing digestibility (Sokrab et al., 2012), and the total phenolic content (Guo et al., 2013; Winarsi et al., 2019). This occurs because the activity of protease, lipase and carbohydrase increases during the germination process, the content of amino acids, fatty acids, and glucose is higher than ungerminated nuts. Glucose,

amino acids, vitamins, and minerals are simple molecules that are easy to digest. More than that, these molecules can be essential nutrients for the growth of lactic acid bacteria (LAB).

Green beans and bean sprouts not only act as a source of nutrition because they contain relatively high amounts of protein and carbohydrates. Both of these foodstuffs also have functional properties that can improve health status, act as immunomodulators. Bean sprouts are antioxidants because they contain a number of flavonoid compounds. The flavonoid compounds include robinin, kaempferol, quercetin, isokertin and kaempferol-7-O-ramnoside. According to Handayani, 2016 in green bean sprout compounds such as flavonoids, carotenoids, and phenolics function as antioxidants because they can donate electrons to stabilize radicals, in the presence of the -OH group attached to the carbon of the aromatic ring, the free radical products of these compounds are stabilized. resonantly and therefore unreactive when compared to most other free radicals so that it can function as an effective antioxidant.

Yoghurt green bean sprout which is made with the role of LAB was also reported by Riaz Rajoka et al. (2017) has other therapeutic properties, such as prophylactic (preventing infection) against several types of intestinal infections, The bacteria in probiotic yoghurt can increase the growth of beneficial gut microbiota and affect intestinal and tissue function through immune system regulation (Maynard et al., 2012).

RESEARCH METHODS

This type of research is experimental research. Quantitative data analysis is immunomodulator test.

Sample.

The research sample was white Sprague Dawley rats, about 3 months old and weighing ± 300 grams, totaling 30 rats. in good health and have normal activities. The treatment group consisted of 5 groups, namely the control group, the negative control group which was given aqua destila 2 ml/200 g BW, the treatment group was given yogurt bean sprouts at a dose of 2 ml/200 g BW, 4 ml/200 gBW and 6 ml/200 gBW. Each group consisted of 5 Sprague Dawley rats plus 1 spare rat in each treatment group.

Material

Materials used are Green beans sprouts, skimmed milk, starter Lactobacillus Bulgaricus/ Lactobacillus Thermophilis and Streptococcus Thermophilus, white sugar 3 kg, yogurt Plan 2 kg, aquades, Na-CMC, pH paper, label Paper, stationery, tissue, filter cloth/gauze and experimental animal (Sprague Dawley Rat)

Research steps:

1. Materials Preparations:

The preparation of research materials included cleaning and sorting green beans, then soaking them in water in a basin for 12 hours and placing them in a place protected from sunlight. After that, the green beans are drained and placed on a tray or basket that has been lined with flannel and strimin. Cover the basket with a black cloth and place it in a place protected from the sun. Green peas are watered every 2 times a day in the morning and evening, the third day the sprouts can be used and the sprouts are taken according to research needs

2. Making Green Bean Sprout Yogurt

Green beans are cleaned and sorted, then soaked with water in a basin for 12 hours and placed in a place that is protected from sunlight and then drained. Green beans are sown on a tampa or basket that has been lined with flannel and strimin cloth, then the basket is covered with a black cloth and placed in a place that is protected from sunlight. Green beans are watered every 2 times a day in the morning and evening, the third day the sprouts can be used and taken according to research needs.

Sorted sprouts, choose the white one, then blanched with hot water (temperature 80°C) for 3 minutes. Sprouts were crushed using a blender with a ratio of sprouts: water, namely

1:7, then filtered using a sieve. The mung bean sprout milk that has been obtained is pasteurized at 90C for 15 minutes while continuously stirring, add skim milk as much as 4% of the volume of milk and 7% sugar, then remove and cool to 40C. Green bean sprout milk that has been cooled is put into a fermentation container as much as 100 ml in each jar. Plain yogurt is added as much as a teaspoon and stirred until evenly distributed. Milk yogurt was fermented for 24 hours at room temperature.

3. Test Animal Acclimatization Stage

A total of 40 male white rats were acclimatized for 2 weeks in the laboratory animal test. Adjustments were made to room conditions, temperature, humidity, feed, drinking water consumption and individual cages. This is useful to familiarize the mice's immune function to their normal conditions.

4. Test Animal Treatment Stage

A total of 25 rats that have undergone a period of acclimatization were divided into 5 groups with 5 rats in each group. Group 1 is a normal control, Group 2 is a negative control given aqua distillate 2 ml/200 gBW, Groups 3, 4 and 5 are a treatment group with yogurt touge with a successive dose of 2 ml/200 g BW, 4 ml/200 g body weight and 6 ml/200 g body weight. Groups 2 to 5 were then treated for 1 week, then their immune system was induced by administering Hepatitis B vaccine at a dose of 18 ul per 200 gBB intramuscularly, 2 times with a distance of 7 days from the first dose of Hepatitis B vaccine. Then the treatment was continued for 14 days so that the total treatment was 30 days. Every 6 days the body weight of the mice was weighed and monitored. After 30 days the rats were terminated and their blood was taken for examination of Hepatitis B Antibodies and Hematology Count Leukocyte Cell Count. In table 2. below shows the body weight of rats for 30 days of treatment.

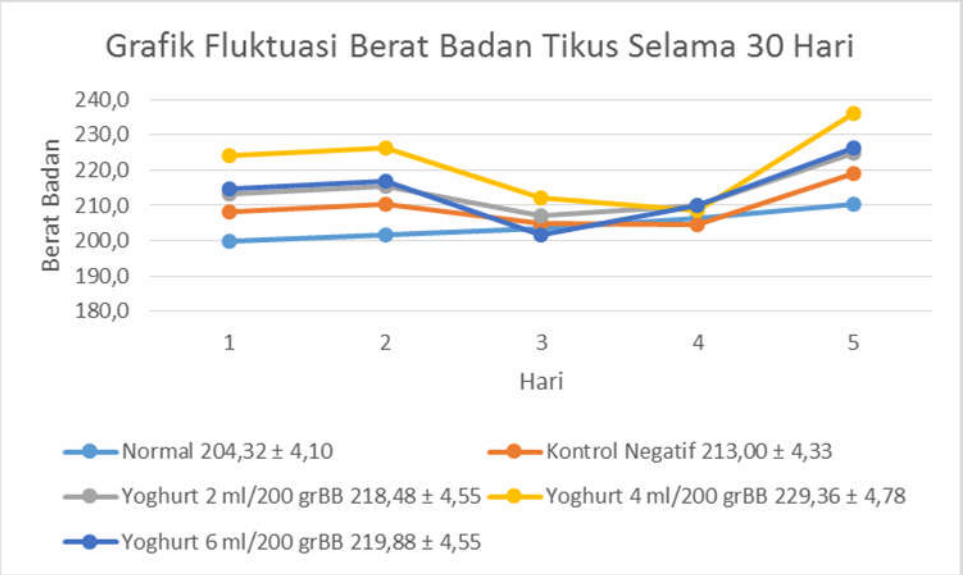
Table 1. Average Rat Body Weight for 30 days of treatment

No	Group	Rat Weight Average (gr)
1	Normal	204,32 ± 4,10
2	Negative kontrol	209,00 ± 6,55
3	Yoghurt 2 ml/200 grBB	214,92 ± 7,19
4	Yoghurt 4 ml/200 grBB	221,40 ± 11,61
5	Yoghurt 6 ml/200 grBB	214,00 ± 9,45

Based on the research data in table 2, it shows that the yogurt touge 4 ml/200 grBB treatment group has the highest body weight, compared to other groups.

During the treatment, the body weight of the rats changed, especially in the 3rd week of treatment. Body weight fluctuations of rats given treatment for 30 days can be seen in graph 1.

In Graph 1 below, it shows fluctuations in the body weight of rats that were treated for 30 day



Based on the research data in graph 1, it can be seen that all treatment groups experienced weight loss in the 3rd week except for the normal group. This is because in the second and third weeks the rats were injected with Hepatitis B vaccine, which affected the rat's food intake.

4. Examination of Leukocyte Count in Rat Blood

After the rats were terminated, the rats were examined for leukocyte hematology using the Improve Neubauer counting chamber method. Table 3 shows the average number of leukocyte cells in rat that were treated for 30 days.

Table 3. The average number of leukocytes in the group of rats that underwent treatment for 30 days

No	Group	Average Leukocyte Count (1000 cells/mm3)
1	Normal	9,46 ± 1,6
2	Negative control	10,82 ± 0,72
3	Yoghurt 2 ml/200 grBB	13,24 ± 3,78
4	Yoghurt 4 ml/200 grBB	15,78 ± 5,26
5	Yoghurt 6 ml/200 grBB	20,1 ± 3,59

Table 3 shows that the highest number of rat leukocyte cells occurred in the group of rats given 6 ml/200 gBB of yogurt, which was 20,100 leukocyte cells. And the lowest was the normal group, namely 9460 leukocyte cells. This indicates the activation of the body's immune system by the presence of immunomodulators in building the immune system as a result of the administration of the Hepatitis B vaccine.

5. Hepatitis B Antibody Test (anti-HBs; HBsAb)

Hepatitis B antibody test (anti-HBs; HBsAb) is performed to assess the immune system (antibodies) produced in response to exposure to hepatitis B virus antigen. body to protect itself. Anti-HBs can be produced by the body through direct exposure to the virus, or through immunization with the Hepatitis B vaccine.

Table 4 below shows the average blood serum HBsAb levels of rat that were treated for 30 days.

Table 4. The mean serum HBsAb levels in the blood of rats treated for 30 days.

No	Group	Average Serum HBsAb Level (ng/ml)
1	Normal	3,91 ± 0,21

2	Negative control	3,96 ± 0,38
3	Yogurt 2 ml/200 grBB	3,99 ± 0,38
4	Yogurt 4 ml/200 grBB	4,33 ± 0,25
5	Yogurt 6 ml/200 grBB	4,72 ± 0,50

Based on the research data in table 4 above, it shows that all treatment groups experienced an increase in the HBsAb antibody titer against the Hepatitis B vaccine. And the increase was more than the normal group HBsAb antibody titer value. So it can be concluded that the administration of Yoghurt Touge was able to increase the value of the HBsAb antibody titer after being given the Hepatitis B vaccine. And the highest was found in the treatment group with 6 ml/200 gBB of yogurt.

The results of statistical analysis showed that the data were homogeneously distributed where the alpha significance value was greater than 0.05, so the test used was the ANOVA test. One-way ANOVA statistical analysis showed that the alpha significance value was less than 0.05, which means that there was a significant difference in the administration of yogurt to the increase in HBsAb antibody titer after being induced with Hepatitis B vaccine.

ANOVA

Treatment Group

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2.401	4	.600	4.611	.008
Within Groups	2.603	20	.130		
Total	5.004	24			

From the results of the ANOVA analysis, it was found that there were differences between the treatment groups. The results of the post hoc analysis showed that there was a difference in HBsAb antibody titers in the control group and the treatment group at a dose of 6 ml/200 g BW. The treatment group with a dose of 6 ml/200 g BW had higher antibody titer values when compared to the normal and negative control groups. So it can be concluded that the administration of bean sprouts yogurt can improve the immunoglobulin system in experimental rats.

Yoghurt is a probiotic drink that is usually produced from animal milk acidified with the help of Lactobacillus. Yogurt can affect changes in the gastrointestinal and systemic immune systems associated with allergies and inflammation (Reid et al., 2013). Microbial cell preparations contain peptides with immunomodulatory activity. Peptides in yogurt have an effect on the immune system, which can induce cellular immune system activity and inhibit inflammatory activity and inhibit excessive immune responses (Grajek et al., 2015). Yogurt will induce the cellular immune system by releasing inflammatory mediators that function as inhibitors of the inflammatory process in the form of cytokines TGF-β and IL-10 secreted by T-regulatory cells (Chiba et al., 2012). This is because in yogurt there are peptides that act as immunomodulators.

Lymphocytes are white blood cells (leukocytes) that are small, round in shape with a diameter of 7-15 m. Lymphocytes are key cells in the process of specific immune responses, to recognize various antigens. Each lymphocyte cell is only able to recognize one antigen so that in the process of immune response, lymphocytes work together to eliminate various antigens that enter the body. Lymphocyte cells consist of B cells and T cells which are both responsible for specific immune responses to recognize antigens through antigen receptors. Lymphocyte cells are also able to distinguish antigens from the body's own components or function as immune controllers.

Proliferation is a fundamental biological function of lymphocytes, namely the process of cell differentiation and division (mitosis). Lymphocytes are single cells that survive well when cultured in simple media. The proliferative response of lymphocyte culture is used to describe lymphocyte function and immune status. (Tejasari, 2010). Consumption of Lactobacillus Lactic Acid Bacteria such as those found in the probiotic drink Yoghurt Bean sprouts can improve the cellular and humoral immune system, including an increase in the population and proliferation of leukocytes, the production of cytokines Interferon (IFN-γ), Interleukin-12, IL-10, Th immune cells. and immunoglobulin (Ig) A, IgE, IgG and IgM.

BAL attached to human epithelial cells can activate macrophages. LAB immune stimulation is through cell wall components, namely peptidoglycan which induces mucosal surfaces. Glucan in the bacterial cell wall will stimulate macrophages to produce IL, increasing the activation of lymphocyte cell proliferation. Lymphocyte cells divide into T lymphocytes and B lymphocytes. T lymphocytes will release interferon, re-activating macrogphages and B lymphocytes in producing

CONCLUSIONS AND RECOMMENDATIONS

Yoghurt bean sprouts are proven to be immunomodulatory which can increase antibodies (immune system) in expreimental rats.Yoghurt Bean sprouts can improve the cellular and humoral immune system, including an increase in the population and proliferation of leukocytes, the production of cytokines Interferon (IFN-γ), Interleukin-12, IL-10, Th immune cells. and immunoglobulin (Ig) A, IgE, IgG and IgM.

REFERENCES

Guo, W. and Beta, T. (2013). Phenolic acid composition and antioxidant potential of insoluble and soluble dietary fibre extracts derived from select whole-grain cereals. Food Research International, 51(2), 518- 525. https://doi.org/10.1016/j.foodres.2013.01.008

Grajek W. A., Olejnik., and A, Sip. (2015). Probiotics, Prebiotics, and Antioxidants as Functional Food. Biotechnology 52 (3) : 665-671

Handayani S, (2016). Determination of Total Phenolic, Flavonoid, and Carotenoid Content of Mung Bean Sprout Ethanol Extract (vVigna Radita) using a UV-Vis Spectrophotometer. Thesis, UIN Alauddin Makassar.

Hari, Kristiyanto D, (2013). Utilization of Breadfruit (Arthocarpus Communis Forst) In Yoghurt With The Addition Of Dragon Fruit Skin Extract (Hylocereus Polyrhuzus) As Natural Dyes. Thesis: Muhammadiyah University, Yogyakarta.

Hasrudin and Rifnatul, (2015). Industrial Microbiology, Bandung: Alfabeta.

Maulana AL, (2010). Effect of Bean sprout (Phaseolus Radiatus) Extract on Paracetamol-Induced Mice Kidney Cell Damage (Mus Musculus). Thesis: Surakarta: Faculty of Medicine, Sebelas Maret University.

Mades Fifendy, Dwi Hilda Putri, Shinta Sari Mariah, (2016) "The Effect of Adding Bean sprouts as a Nitrogen Source on the Quality of Nata De Cocoa", Scientific Journal, Vol. III, No. 2, 2016, p.165

Pattorn S, Horimoto Y, Hongsprabhas P, and Yada, (2012). Influence of aggregation on the antioxidative capacity of milk peptides. int. Dairy J. 25:3–9.

Persagi, (2017). Indonesian Food Composition Table. Jakarta: PT Elex Media Komputindo
Reid G, Jass J. Sebulsky MT. McCosmick J K. 2013. Potential use of probiotics in clinical practice. Cline microbial. Rev 16: 658-672

Samichah, (2014). Antioxidant Activity and Organoleptic Acceptance of Carrot Juice Yoghurt (Daucus carrota L.). Research Articles. Nutrition Program, Faculty of Medicine, Diponegoro University: Semarang.

Sokrab, A.M., Ahmed, I.A.M. and Babiker, E.E. (2012). Effect of germination on antinutritional factors, total, and extractable minerals of high and low phytate corn (Zea mays L.) genotypes. Journal of the Saudi Society of Agricultural Sciences, 11(2), 123-128. https://doi.org/10.1016/j.jssas.2012.02.002
Sopandi and Wardah, (2014). Food Microbiology. Yogyakarta: Andi Offset.

Winarsi, H., Sasongko, N.D. and Purwanto, A. (2016). Germinated-soy milk in suppressing inflammation and oxidative stress in blood plasma and breast milk of lactating mothers. International Food Research

Winarsi, H., Septiana, A.T., Kartini. and Hanifah, I.N. (2019). Fermentation of lactic acid bacteria improving phenolic and fibre content of red kidney bean sprouts milk (Phaseolus Vulgaris L.) yogurt, a functional drink for obesity. Jurnal Gizi dan Pangan Soedirman, 3(1), 64-75. https:// doi.org/10.20884/1.jgps.2019.3.1.1526