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Increased Levels Of Fermented Soy Milk Amino Acids And Effectivenes Helping Reduce Uric Acid Serum In Male White Rats

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***Abstract*— Soybean is one of the most important foodstuffs for the people of Indonesia, which is consumed to meet the nutritional needs of protein. Soybeans are consumed by the public as a side dish and snack. Soy milk is a form of processed soybean. Previous research has shown that tempeh, which is a form of fermented soybean, can reduce purine levels in soybeans, and can increase nutritional value, especially amino acid content. The purpose of this study was to determine the increase in amino acid content of fermented soy milk with the help of Lactobacilus Kasei bacteria, and its effect in helping to reduce serum uric acid levels in the blood of male white rats. Fermented soy milk is made of 250 grams of soybeans and squeezed in 5 liters of water, then heated. Lactobacilus kasei is used as a battery for fermentation and 500 grams of palm sugar is used as a source of nutrition for bacteria. The test to reduce uric acid levels was carried out in vivo by giving male white rats drinking as much as 10 mL, 20 mL, 30 mL, 40 mL, 50 mL for 14 days, then taking the blood through the tail vein, then measuring the uric acid levels using the strip method. test with the GCU easy tooch tool. To increase uric acid levels in male white rats was done by induction with chicken's liver. The results of measuring the levels of amino acids in the fermentation of soy milk increased between 1-2% with the following levels: Lysine 4.7%, Histidine 2.3%, Arginine 4.9%, Asparkate 7.8%, Treonine 2.9%, Serine 2.9%, Glutamate 14.2% , Proline 3.8%, Glycine 3.7%, Alanine 2.8%, Cystine 1.6%, Valine 3.2%, Methionine 1.2%, Isoleucine 3.8%, Leucine 5.9%, Tyrosine 2.7% , Phenylalanine 3.3%. Testing of uric acid levels in the blood of male white rats after fermented soy milk with various volume series were as follows: 6.70 mg/dL ± 1.16; 5.88 mg/dL ± 0.81; 4.63 mg/dL ± 0.92; 4.18 mg/dL ± 0.71; 4.16 mg/dL ± 0.96. Based on the results of the study, it can be concluded that fermented soy milk with the help of lactobacillus kasei bacteria can increase amino acid levels ranging from 1-2%, and the provision of drinking fermented milk can help reduce blood uric acid levels in male white rats with blood uric acid levels by 4.16 mg/dL ± 0.96.**

Keywords—Fermentation, lactobacillus casei, amino acids, potassium oxonate, uric acid

I. INTRODUCTION

Advances in science and technology have resulted in changes in human life patterns. Modern lifestyles have brought humans into a life that is instantaneous, practical and fast causing a disease transition in society from infectious diseases to degenerative diseases. This has resulted in an increase in degenerative diseases, one of which is hyperuricemia or gout [13].

Uric acid is the final catabolism of purines aided by the enzyme xanthine oxidase. Uric acid is transported to the kidneys by the bloodstream and excreted in the urine. The kidneys will keep the uric acid level in the blood so that it is always in a normal state. In an excess of uric acid will be accumulated in the body resulting in an increase in uric acid levels, causing hyperuresemia [3].

Hyperuresemia in an advanced stage causes gout. Gout is a disease that affects the life activities of more than 1% of adults in the world. This disease affects human joints causing pain, warmth and swelling in the joints and results in joint tissue damage [9]. One of the ways to reduce uric acid levels in the body is by inhibiting the enzyme ksantin oxidase, such as allopurinol, to reduce uric acid levels in the blood [15]. Allopurinol is a drug used to treat hyperuresemia and gout. On long-term use and large doses allopurinol causes several side effects such as allergic reactions, indigestion, bone marrow depression and aplastic anemia [6].

Soybean is one type of food that is widely consumed by Indonesians. Soybeans are generally consumed directly or in their processed form. Soy milk, which is a type of processed soybean, can increase uric acid levels due to high levels of purines [4]. Fermentation of legumes can increase amino acid levels by 1% to 5% of the protein breakdown process. In addition, the fermentation process can also reduce the levels of purines contained in beans [11]. The fermentation process causes protein to be more easily digested and improves the body's metabolic processes, which can help reduce uric acid levels in the blood [7].

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II. METHOD

A. Making fermented soy milk

Soy milk is made from 500 grams of soybeans with 5 liters of distilled water and 500 grams of brown sugar, then

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heat it to a boil while continuing to stir and cool. After chilling put it in the sterilized fermenter tube and add the Lactobacillus s.p bacteria then ferment it for 5 days.

B. Evaluation of Antihyperuricemic Activity

A total of 40 male white rats weighing 150-200 grams were acclimatized to suit the experimental conditions. Animals were grouped into positive controls, negative controls (I and II) and 5 other groups that also received high-purine diets as hyperuricemia inducers. High-purine foods are made in homogeneous form with a composition of 100 mg of fresh chicken liver [14]. Fermented milk with a dose of 10 mL, 20 mL, 30 mL, 40 mL, 50 mL/day, positive control used allopurinol 10 mg/kgBB given after high-purine feeding and negative control I only given chicken liver without any treatment, while negative control was given II was given chicken liver as an inducer and soy milk without fermentation. This treatment is given for 13 consecutive days. On the 14th day, the animal's blood was drawn through its tail to measure the serum uric acid levels with a uric acid test strip (easy tooch GCU), which then obtained the blood uric acid levels expressed in mg/dL.

C. Protein Content

Protein content measurements were carried out using the Kjeldahl micro method. Samples were weighed as much as 1-2 grams, then put into a 100 mL Kjeldahl flask, then added 0.25 grams of selenium and 3 mL H_2SO_4, concentrated. The sample is digested at 410 °C for approximately 1 hour until the solution is clear and then cooled. Once cool, 50 mL of distilled water and 20 mL of 40% NaOH are added to the Kjeldahl flask, then the distillation process is carried out with a distillation temperature of 100 °C. The distillation results are collected in a 125 mL Erlenmeyer flask containing a mixture of 10 mL of 2% boric acid (H_3BO_3) and 2 drops of bromcherosol green-methyl red indicator which is pink in color. After the distillate volume reaches 40 mL and is bluish green, the distillation process is stopped. Then the distillate is titrated with 0.1 N HCl until a pink color changes. The volume of the titrant is read and recorded. The blank solution is analyzed as an example. The percentage of nitrogen in the sample can be calculated using the following formula [1]:

$$\% N = \frac{(mL\ Sample - Blank)\ \text{x Normalitas x } 14{,}007\ \text{x } 100}{mg\ Sample}$$

Protein content is calculated using the following formula:

% Protein = % N x F

Where F = conversion factor = 100 /% N in the sample protein. The conversion factor depends on the type of sample.

D. Amino Acid Analysis

Amino acids were analyzed using HPLC. The principle of this amino acid analysis is that amino acids from protein are liberated by hydrolysis with 6N HCl. Hydrolyzate is dissolved with sodium citrate buffer and each amino acid will be separated using HPLC. Before the hydrolysis process is carried out, first the protein extraction is carried out using the Kjeldahl method [2].

III. RESULT

Soy milk is one of the processing products which is the result of extraction from soybeans. Soy milk is a highly nutritious drink, especially its protein content. However, soy milk also contains high levels of purines so that it can increase uric acid levels in the blood. Fermenrasi is a food preservation technique. In addition, fermentation can be used to increase amino acid content by breaking down protein into a simpler form so that it is easily digested and improves metabolism. Fermentation can reduce purine levels in nuts.

Amino acids are the main components of protein, and are divided into two groups, namely essential and non-essential amino acids. Essential amino acids cannot be produced in the body so they often have to be added in the form of food, while non-essential amino acids can be produced in the body [12]. The amino acid content in soy milk is presented in Table 1.

Table 1. Amino acid composition of soy milk before and after fermentation.

Amino Acid	Before Fermentation	After Fermentation
Lisin	3,3 %	4,7 %
Histidin	1,4 %	2,3 %
Aginin	4 %	4,9 %
Asparkat	7,1 %	7,8 %
Treonin	2,1 %	2,9 %
Serin	2,5%	2,9 %
Glutamat	11 %	14,2 %
Prolin	2,7 %	3,8 %
Glisin	3,1 %	3,7 %
Alanin	2,3 %	2,8 %
Sistin	0,8 %	1,6 %
Valin	2,6 %	3,2 %
Metionin	0,86 %	1,2 %
Isoleusin	3,3 %	3,8 %
Leusin	4,7 %	5,9 %
Tirosin	2,2 %	2,7 %
Fenilalanin	3 %	3,3 %

In testing the fermentation of soy milk on serum uric acid levels in male white rats. Selection of male sex is intended for simplicity in research and to avoid the influence of hormonal factors. The hormone estrogen found in female subjects will increase the excretion of uric acid from the body so that the risk of gout is relatively lower than that of male subjects [5]. Provision of fermented soy milk with different dosage variations for each treatment, namely a dose of 10 mL/day, 20 mL/day, 30 mL/day, 40 mL/day, and 50 mL/day, days are given for 13 days. As a comparison, negative control was given by giving chicken liver feed without any kind of care and positive control by giving allopurinol 10 mg/kg BB given after giving high-purine food.

In this study, giving chicken liver for 13 days aims to obtain hyperuricemia conditions in each treatment given fermented soy milk with five dose variations as a treatment factor, namely 10 mL/day, 20 mL/day, 30 mL/day, 40 mL/day and 50 mL/day. The results of measuring uric acid levels in male white rats before induction using chicken liver and after being given treatment on the 14th day can be seen in Table 2.

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Table 2. Average results of measurements of rat uric acid levels before induction and after treatment.

Group	Before Induction (mg/dL)	After Treatment (mg / dL)
Positive control	4,36 ± 1,08	3,56 ± 0,26
Negative control I	4,50 ± 0,72	10,8 ± 1,14
Negative control II	4,43 ± 1,12	12,2 ± 0,86
Dose 10 mL/day	4,86 ± 0,85	6,70 ± 1,16
Dose 20 mL/day	4,52 ± 0,85	5,88 ± 0,81
Dose 30 mL/day	4,54 ± 0,31	4,63 ± 0,92
Dose 40 mL/day	4,74 ± 0,42	4,18 ± 0,71
Dose 50 mL/day	4,91 ± 0,56	4,16 ± 0,96

From the table above, it can be seen that there was a significant increase in uric acid levels after being given chicken liver for 13 days, this was shown in negative control I which was given chicken liver as an inducer of hypererusemia, there was a significant increase in the initial uric acid level before induction of 4.50 mg. / dL ± 0.72 increased to 10.8 mg / dL ± 1.14 on day 14, this indicates that the induction was successful. The increase in uric acid levels was higher in negative control II, which was given chicken liver as an inducing agent and soy milk without fermentation every day, this proved that soy milk, which is one type of food high in urine, can increase uric acid levels in the blood.

A decrease in uric acid levels occurred in the test group that was given orally fermented soy milk for 13 days. The decrease began to occur from the smallest dose given 10 mL/day and continued to decrease with increasing doses of fermented soy milk, this shows that fermented soy milk can help reduce levels of land acid. The fermentation process can break down proteins into simpler so that they are easily digested and increase the body's metabolic processes. The highest decrease in uric acid levels occurred in positive controls who were given allopurinol at a dose of 10 mg / KgBB with uric acid levels of 3.56 mg/dL ± 0.26. Allopurinol is a gout or gout drug with a mechanism of inhibiting purine formation through inhibition of the xanthin oxidase enzyme. Allopurinol is the only uricostaticum currently used therapeutically, which works to reduce uric acid build-up. Meanwhile, those that work to increase uric acid elimination are called uricosurics [8]. Allopurinol is a substrate of xanthine oxidase and is eliminated through the kidneys primarily as oxypurinol (often called the wrong term, alloxanthine) [10]. Allopurinol and oksipurinol, inhibit canteen and uric acid, which in low doses the inhibitory mechanism is competitive and in high doses it works uncompetitively. A decrease in blood uric acid levels can be seen in Figure 1.

IV. CONCLUSION

Based on the research that has been done, it can be concluded: The fermentation process assisted by Lactocillus s.p bacteria can increase the levels of amino acids in soy milk. Fermented soy milk can help reduce blood uric acid levels.

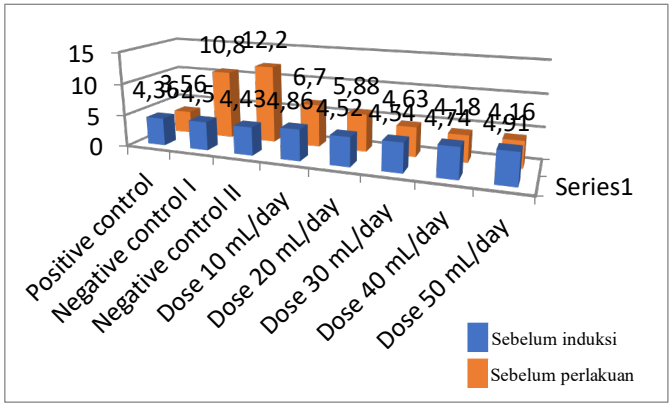

Figure 1. Uric acid levels before induction and after treatment

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