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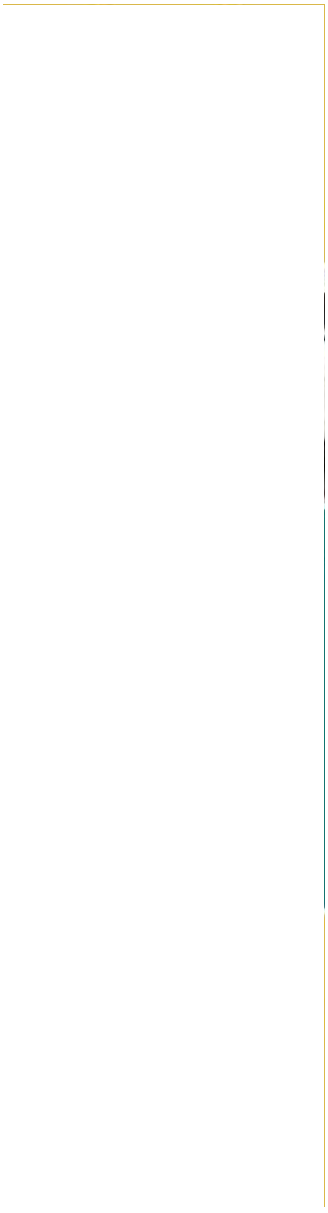

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Preface

The Second International Conference on Food and Agricultural Sciences (2nd ICFAS 2023) December 12 – December 14, 2023

Research Organization for Agriculture and Food National, Research and Innovation Agency (BRIN) Hybrid from Yogyakarta, Indonesia

PREFACE

The Second International Conference on Food and Agricultural Sciences (2nd ICFAS 2023) started as part of the research and dissemination activities of the Research Organization for Agriculture and Food, Research and Innovation Agency (BRIN) with its first event in 2022. The theme of the 2nd ICFAS 2023 is "The Future Innovation and Technology in Food and Agriculture Sustainability to Face Climate Change". The ICFAS 2023 serves as a platform for researchers, scientists, lecturers, engineers, scholar students, and practitioners interested in the advanced technologies of agriculture and food including food technology and processing, pre- and post-harvest technologies, food and nutrition securities, climate change, smart farming, agriculture and food supply chain system and related topics. The conference aimed to create an environment where diverse perspectives converge, fostering a rich tapestry of ideas that can drive meaningful progress in relevant fields or industries.

In today's rapidly evolving world, the exchange of knowledge has never been more crucial. The challenges we face are multifaceted, and it is only through collective intelligence, interdisciplinary collaboration, and a commitment to excellence that we can navigate the complexities ahead. The ICFAS 2023 is designed to be a nexus for such collaboration, where experts, scholars, and practitioners converge to share their expertise and experiences. Over the three days (12-14 December 2023), The conference has curated a program that features (keynote speakers, invited speakers, panel discussions and workshops) to explore the latest trends, address pressing issues, and inspire new thinking in food and agriculture scopes. We encourage you not only to actively participate in these sessions but also to engage in informal discussions during breaks, fostering connections that extend beyond the confines of this conference.

The success of this conference is not only measured by the wealth of information shared but also by the relationships forged and the potential collaborations that may emerge. We would like to express our deep appreciation to all the sponsors (Institut Pertanian STIPER Yogyakarta, PT Wiralab Analitika Solusindo, PT Bayer Indonesia - BCS, PT Fajar Mas Murni, CropLife, PT Elo Karsa Utama, PT Corteva Agriscience Indonesia, PT ITS Science Indonesia, ID Food, and Danone Indonesia), partners (Faculty of Agricultural Technology, Faculty of Agriculture, Faculty of Biology, Faculty of Animal Husbandry, and Study Program of Biotechnology - Graduate School, Universitas Gadjah Mada; Faculty of Science and Technology, UIN Sunan Kalijaga; Faculty of Agricultural Technology, STIPER Agricultural Institute Yogyakarta; Faculty of Mathematics and Natural Sciences, Universitas Islam Indonesia; Faculty of Industrial Technology, Universitas Ahmad Dahlan; Faculty of Science and Technology, Universitas Aisyiah; Faculty of Biotechnology, Universitas Atmajaya Yogyakarta; Faculty of Halal Industry, Universitas Nahdhatul Ulama; Faculty of Agricultural, UPN Veteran Yogyakarta; Faculty of

Mathematic and Natural Sciences, Yogyakarta State University; Indonesian Biotechnology Consortium) organizers, presenters, and, above all, to the participants, for the priceless contributions of time, expertise, and enthusiasm that have contributed to the success of this conference.

Sincerely,

Satriyo Krido Wahono, Ph.D.
Chairman

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Changes in lipid profiles of hypercholesterolemic rat (*Rattus norvegicus*) treated with iles-iles synbiotic effervescent tablets

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Changes in lipid profiles of hypercholesterolemic rat (*Rattus norvegicus*) treated with iles-iles synbiotic effervescent tablets

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Abstract. The development of innovations in the use of functional food is growing, especially in the health sector. Synbiotic products are developed by combining prebiotics with probiotics. Synbiotic effervescent tablets provide prebiotic and probiotic compounds as supplements so they can be consumed and provide healthy effects. This study aims to determine the effect of giving synbiotic effervescent tablets from iles-iles tubers on lipid profiles and determine the lowest dose of synbiotic effervescent tablets in reducing cholesterol levels in blood rats. This study used the Randomized Complete Block Design as the experimental design, with a dose of tablet treatment 0.045 g/day/200 g rat body weight (half of normal dose), 0.09 g/day/200 g rat body weight (normal dose), and 0.18 g/day/200 g rat body weight (twice of normal dose). Rats given distilled water without being treated with tablets were used as controls. The lipid profiles (total cholesterol, triglycerides, HDL, and LDL) of the rats were measured after the treatment. Body weight changes were also monitored throughout the study. Treatment using synbiotic effervescent tablets at all doses for 28 days can lower total cholesterol, triglycerides, and LDL and increase HDL. The highest cholesterol reduction (49.94%) was found in rats given tablet doses twice the normal dose for 28 days.

1. Introduction

Hypercholesterol is a disease caused by an imbalance in cholesterol intake from food and synthesis in the body [1]. This disease is characterized by an increase in cholesterol levels beyond normal, causing atherosclerosis, which is a blockage in the arteries due to the swelling of the artery walls [1]. The incidence rate of hypercholesterol in Indonesia is 9.3% at the age of 25-34 years and increase15.5% at 55-64 years [2]. Hypercholesterol has also been reported to be a cause of coronary heart disease (CHD), with an incidence rate of 4.4 million or 18% worldwide [2]. Hypercholesterol can be causedby various aspects of unhealthy food intake and fitness problems to maintain the health of the patient's body [3]. There are several ways to overcome hypercholesterolemia through dietary interventions and incorporating natural foods that can help lower cholesterol levels [4]. The natural food ingredients are iles-iles tubers (*Amorphophallus oncophyllus*), which contain glucomannan compounds thought to function as lowering cholesterol levels [5].

Glucomannan or konjac mannan is a heteropolysaccharide consisting of D-mannose and D-glucose in a ratio of 1.6:1 with a combined β (1.4) [6]. The iles-iles tubers contain 66.89 % of glucomannan [7]. Glucomannan powder, which is soluble fiber and insoluble fiber [8]. Soluble fiber will lower cholesterol levels by binding it in the digestive tract and then carrying it out of the body [9].

Consuming an adequate amount of fiber is important for maintaining good digestive health and preventing constipation [9].

Iles-iles tubers have a high potential source of prebiotics because they contain undigested carbohydrates, namely glucomannan, starch, and crude fiber [10]. The glucomannan flour will be utilized produce synbiotic microcapsules by encapsulating prebiotics with probiotics. These microcapsules will then be turned into effervescent tablets as carriers of glucomannan compounds [11]. Glucomannan serves as a source of prebiotics for bacteria in the large intestine [10].

Effervescent tablets are one form of tablet preparation that is made by pressing active ingredients with a mixture of organic acids, such as citric acid or tartaric acid, and sodium bicarbonate [11]. The chemical reaction starts when the acid reacts with other substances, producing carbon dioxide and water. The reaction is fairly quick and usually lasts within a minute or less. Effervescent tablet innovation needs to be developed to add more selling value and function, including the development of effervescent tablets containing synbiotic microcapsules. Therefore, for effervescent tablet products, it is necessary to conduct in-vivo trials on living things, one of which is the treatment of experimental animals regarding the effect of effervescent tablets in reducing cholesterol levels in the blood.

The problem is a little information about the application of glucomannan in effervescent tablets as a binder and their effect on cholesterol levels. In addition, the administering of doses given to animals is not yet known. The study aims to determine the effervescent dose levels for hypercholesterolemia rats, and its cholesterol-lowering effectiveness using rats as test subjects. Therefore, this study was tested on white rats with predetermined dose variations so that various types of doses would get the best dose so that it could be converted to humans. This research aims were to study the effect of givingsynbiotic effervescent tablets from iles-iles tubers on reducing cholesterol levels in blood cells, (2) to get the lowest dose of effervescent tablets in decreasing cholesterol levels in blood cells.

2. Materials and methods

The material used in this study were white rat, *Lactobacillus casei* FNCC 0090 cells, pork oil, and aquadest (obtained from the Food and Nutrition Laboratory, Universitas Gadjah Mada). Iles-iles tubers from the Sentolo Market, protective material (Arabic gum), and Comfeed AD-2 from the Tekun Jaya store. The Effervescent tablet ingredients including citric acid, tartaric acid, sodium bicarbonate, PEG 6000, mannitol, and stevia obtained from Biotechnology Laboratories, Faculty of Pharmacy Universitas Gadjah Mada.

2.1. Preparation of synbiotic effervescent tablets

Synbiotic effervescent tablets are produced by combining synbiotic microcapsules made from biomass *Lactobacillus casei* FNCC 0090 cell, glucomannan iles-iles, and Arabic gum with effervescent tablet ingredients such as citric acid, tartaric acid, sodium bicarbonate, PEG 6000, mannitol, and stevia. The formula and method of making synbiotic effervescent tablets refer to Ngatirah methods published in patent news [12].

2.2. Animal ethics and management

Before being given treatment feed, rats were adapted to a standard diet for seven days to be applied to the environment. Rats are kept in cages with temperatures kept ± 25°C. The handling of test creatures is following widely recognized conventions. The study was approved by the Medical and Health Research Ethics Committee (MHREC) of the Universitas Gadjah Mada (approval No: KE/FK/0218/EC/2022, approval date: March 2, 2022).

2.3. Preparation of hypercholesterolemic rat

White rats with as many as 24 heads weighing 150-250 grams were randomly divided into four groups (three treatments and one as a control). Furthermore, all four groups of rats were fed a diet high in cholesterol, consisting of lard and egg yolk, to induce hypercholesterolemia with levels up to 170 mg/dL. Then, the four groups of mice weighed and analyzed cholesterol levels in the blood.

2.4. Treatment of hypercholesterolemic rats

White rats were divided into four groups, namely three groups for treatments and one group as a control. Three groups of hypercholesterolemic rats were treated with synbiotic effervescent tablets.The three treatments are the number of effervescent tablet doses (half normal dosage 0.045g/200g of rat weight (dose A), normal dosage 0.09g/200g of rat weight (dose B), and twice of normal dosage 0.18g/200g of rat weight (dose C). The calculation of the normal dose of effervescent tablets to rats is the dosage of effervescent tablets for humans (5 g) x 0.018 (conversion factor) = 0.09g/200g weight ofrat. Each group consisted of 6 rats and was then given 200 g of AD-2 feed.

Each group of rats was given effervescent tablets referring to a predetermined dose in which there was a dose of synbiotic effervescent tablets in white rats given dose A 0.045 g/rat/day, dose B 0.09 g/rat/day and dose C 0.18 g/rat/day. The control rats were not given synbiotic effervescent tablets. The synbiotic effervescent tablets should be administered once daily and observed for one month. The administration of symbiotic effervescent tablets dissolved in water and taken to rats. During the treatment, the rats were fed (comfeed AD-2) as much as 10% of body weight and were given ± 200 ml drink per group. After being given the appropriate dose, Rats were weighed weekly, and took blood samples to analyze their blood profiles. Blood collection is done through the temples of the eyes, pierced with a pipette every seven days. Then, the sample is analyzed for lipid profiles (triglycerides, cholesterol, HDL, and LDL) using the enzymatic–calorimetric method CHOD–PAP.

3. Results and discussion

3.1. Changes in triglycerides in the blood of rats

A triglyceride (TG) particle comprises of a glycerol spine esterified with three fatty acids. Triglycerides are the major dietary fat. They are hydrolyzed within the intestine by lipases to fattyacids and monoglycerides. Normally, the levels triglycerides in rats are 26-145 mg/dL. Changes in triglycerides in the blood of rats for 28 days are shown in Figure 1.

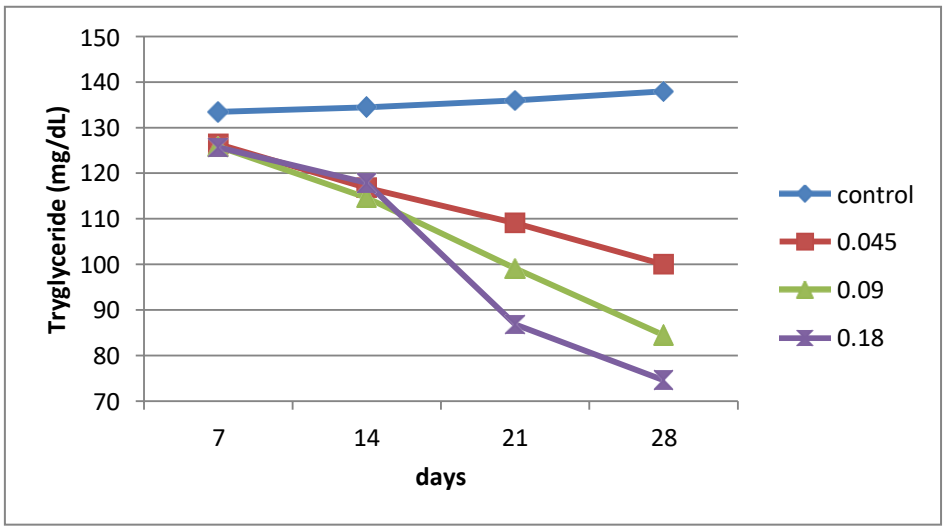

Figure 1. Changes in triglycerides in the blood of rats

Administration of synbiotic effervescent tablets on Dose A, Dose B, and Dose C caused a significant decrease in triglycerides for four weeks (Fig 1.) The decrease in triglycerides shows a different percentage decrease in 28 days. The percentage reduction in Dose C has a higher value of 40.60% compared to Dose A 20.88% and Dose B 32.85%. In the control, treatment did not experience a decrease in triglycerides. The effervescent tablets are responsible for reducing triglyceride levels. These tablets contain *L. casei*, which produces short-chain fatty acids during fermentation. These compounds compete with HMG CoA to bind to the enzyme HMG CoA reductase, inhibiting

cholesterol synthesis [13],[14]. Propionate is a type of short-chain fatty acid that has the ability to impact cholesterol levels. Propionate can inhibit acetate incorporation to plasma triacylglycerol and also tends to inhibit acetate incorporation to plasma cholesterol [15]. This will result in decreased cholesterol synthesis because acetate is a precursor in the formation of cholesterol. Triglycerides are fat in the blood, which can cause blockage in arteries if their levels are too high. These are carried by lipoproteins that are also involved in transporting cholesterol. Unlike cholesterol, stored in liver tissue or blood vessel cells, triglycerides are stored under the skin. The results of the table above have decreased so that it can have a good effect on the increase in HDL (High-Density Lipoprotein) levelsin blood vessels, in contrast, if the results of triglycerides increase, high triglyceride levels will form VLDL and will form LDL, which is very easily oxidized damaging HDL so that it will aggravate cholesterol levels in blood vessels.

3.2. Changes in cholesterol in the blood of rats

Cholesterol is a fat compound produced by various cells in the body. About a quarter of the cholesterol produced in the body is produced by liver cells. Regularly, the levels of added cholesterol within the rat are 10-54 mg/dL. Hypercholesterolemia is a condition where cholesterol levels in the blood are higher than normal. Changes in cholesterol in the blood of rats for 28 days are shown in Figure 2.

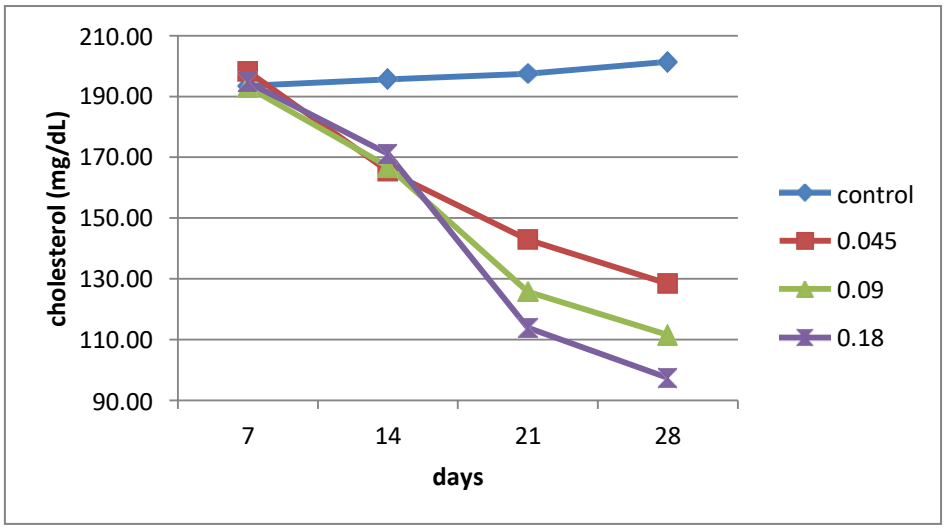

Figure 2. Changes in cholesterol in the blood of rats

Administration of synbiotic effervescent tablets on Dose A, Dose B, and Dose C caused a significant decrease in cholesterol in the blood of rats for four weeks (Fig 2.). The decrease in cholesterol shows a different percentage decrease in 28 days. The higher the dose, the higher the decrease in cholesterol levels. It is due to effervescent tablets contain soluble fiber glucomannan. In addition, the role of glucomannan as a prebiotic can improve the growth of microorganisms in the intestine [15], especially *Bifidobacteria*, reduce the intensity of enteric pathogenic bacteria, regulate immunoreactions [16], and increase the integrity of the mucosa in the intestine [17], [18]. Glucomannan can promote the growth of *L. casei* in the intestine [19]. Probiotic bacteria can lower cholesterol by breaking down bile salts through deconjugation [20]. Bacteria-producing Bile Salt Hydrolase (BSH) enzymes can hydrolyze or break the C-24 N-acyl amide bond formed between bile acids and amino acids in conjugated bile salts [20].

According to Gallaher et al., glucomannan can lower liver cholesterol levels through viscosity that blocks or reduces cholesterol levels or fat accumulation [21]. Meanwhile, glucomannan compounds have hypercolloidal properties, which can thicken to bind excess fat intake that enters the body through food. In other words, it affects the absorption of fats attached to fatty acids, cholesterol, and bile salts [21]. Fatty acids and cholesterol bound to fiber cannot form the micelle required for fatty

acid absorption to pass through the unstirred water layer through enterocytes. As a result, fat bound to fiber cannot be absorbed and will be passed on to the large intestine and excreted through feces [22].

Bile acid reduction is one factor that can lower cholesterol. The compound forming bile acids is cholesterol. The body excretes cholesterol through two main pathways: converting cholesterol into bile acids and through catabolism into steroid hormones and neutral sterols such as cholestanone and coprosterol [23]. The conversion of cholesterol to bile acids is a cyclical process. When cholesterol is converted into bile acids, it gets reabsorbed by the liver and converted back into cholesterol. As a result, the cholesterol level in the body remains relatively constant and does not decrease. Secondary forms of bile acids formed in the form of deoxycholic or cytosolic acids cannot be reabsorbed by the liver [24]. Bile salts that are not absorbed will be excreted with feces. Bile acids conjugated with eithertaurine or glycine, mixed with pancreatic juice, and in the ileum, bile acids bind to sodium or potassium salts. These conjugated bile acids are then reabsorbed and carried back to the liver [24]. If the level of bile acids remains constant, the body won't utilize the cholesterol. The only way to lower cholesterol levels is by using the body's homeostatic system. Bacteria in the ileum can consume bile acids and reduce their amount. Probiotic bacteria can help reduce cholesterol levels [13]. This ability comes from substances that inhibit cholesterol-forming enzymes. During growth, bacteria absorb cholesterol into their cells. If the concentration of bile acid in the body decreases, bile acid synthesis will use cholesterol. With the increasing number of bacteria that can use bile acids, it becomes more beneficial in lowering cholesterol levels, as the cholesterol is utilized to maintain the concentration of bile acids to be present in constant amounts.

3.3. Changes in low-density lipoprotein (LDL) in the blood of rats

LDL cholesterol is a fat that circulates within the blood, moving cholesterol around the body to where it is required for cell repair and keeping it interior of supply route dividers. The level of normal LDL cholesterol in rats was 7–27 mg/dL. Changes in low-density lipoprotein (LDL) in the blood of rats for 28 days are shown in Figure 3.

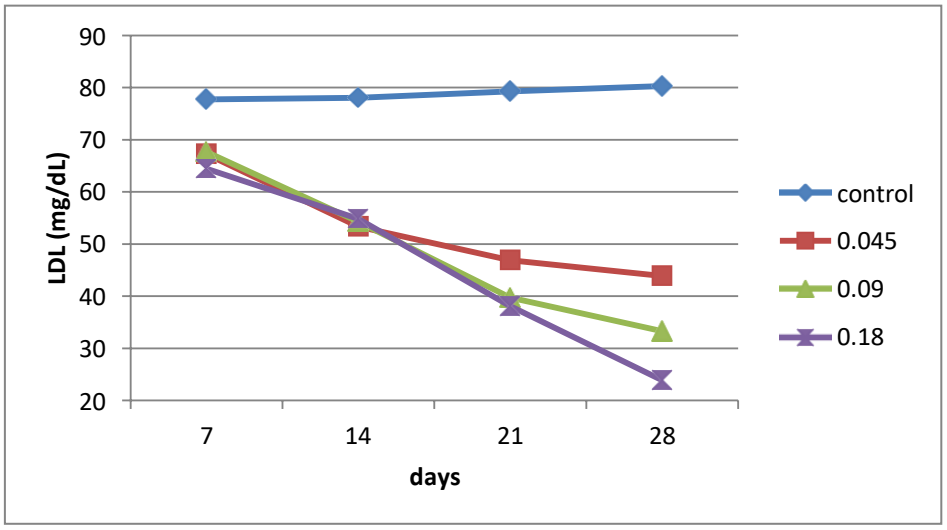

Figure 3. Changes in low-density lipoprotein (LDL) in the blood of rats

The results of the analysis of LDL (Low-Density Lipoprotein) levels in white rats for four weeks after being given effervescent tablets are shown in Fig. 3. Administration of synbiotic effervescent tablets on Dose A, Dose B, and Dose C caused a significant decrease in LDL in the blood of rats. The decrease in LDL (Low-Density Lipoprotein) levels shows a different percentage decrease. The percentage reduction in Dose C has a higher value of 138.2% compared to Dose A 56.8% and Dose B 86.6%. In the control treatment, LDL (Low-Density Lipoprotein) was noted decreased but slightly

increased. Increased levels of LDL (Low-Density Lipoprotein) in the blood will disrupt cholesterol metabolism so that there is a buildup of fat in the fat layer.

Based on Fig 3. it can be seen that the higher the dose, the higher the decrease in LDL cholesterol levels. Triglyceride levels affect LDL levels. When you consume food, if the amount of triglycerides in your body increases, it can cause an increase in the concentration of LDL. Decrease in LDL (Low-Density Lipoprotein) cholesterol levels due to after the process of digestion, food will form acetyl CO-A which enters the Krebs cycle to form ATP [25], so that the process of cholesterol formation and transportation throughout the body will decrease, which results in LDL as a means of transportation is not formed, so LDL levels decrease. High LDL levels lead to high levels of intimal LDL. Furthermore, intimal LDL will undergo oxidation, pull monocytes from the blood circulation, and change phenotypically into macrophages. An increase in oxidized LDL on artery walls, accompanied by the formation of foam cells, will develop into fat plates [26]. Consuming foods high in saturated fat and cholesterol will reduce formation of LDL receptors, leading to high LDL levels. Increased cholesterol will interfere with the metabolism of LDL in the blood [27].

3.4. Changes in high-density lipoprotein (HDL) in the blood of rats

HDL is cholesterol which functions to clean excess dangerous cholesterol in the blood and bring it back to the liver and excreted from the body. Normal HDL cholesterol level in rat blood plasma is ≥35 mg/dL. Changes in high-density lipoprotein (HDL) in the blood of rats for 28 days are shown inFigure 4.

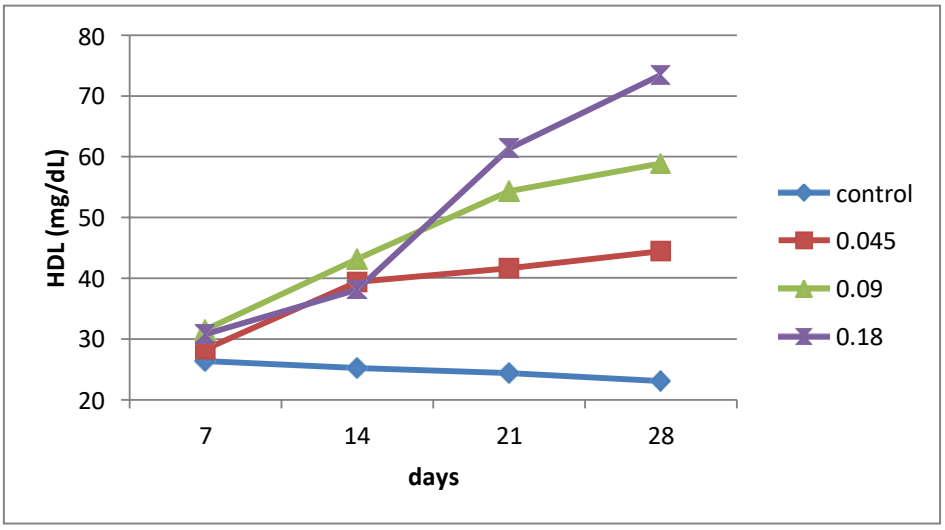

Figure 4. Changes in high-density lipoprotein (HDL) in the blood of rats

The HDL (High-Density Lipoprotein) analysis of white rats for four weeks shown in Fig 4. Administration of synbiotic effervescent tablets on Dose A, Dose B, and Dose C caused a significant increase in HDL in the blood of rats. The HDL levels show a different percentage increase: dose C has a higher value of 138.2% compared to doses A and B. In the control treatment, HDL (High-Density Lipoprotein) levels were not increasing and even tended to decrease.

Based on Fig 4. it can be seen that the higher the dose, the higher the increase in HDL cholesterol levels. It will affect the percentage change in HDL levels in white rats. HDL is a component of lipoprotein Apo-A, which is good cholesterol because it can break down fatty cholesterol in the blood into bile or bile [28]. The high protein content in HDL aids in fat breakdown, preventing artery blockage [29].

In the analysis of cholesterol levels, the results of a decrease were obtained so that the results were interconnected with an increase in HDL levels. Based on the research results, HDL can act as an antioxidant and antithrombotic in addition to its role in lipid transport in the blood [30]. HDL is also

important for maintaining the normal condition of vascular endothelium, inhibiting cell apoptosis, and playing a role in the repair of damaged endothelium. HDL is thought to have antiatherogenic effects, including inhibiting the oxidation of LDL, inhibiting endothelial inflammation, increasing endothelial nitric oxide production, increasing prostacyclin bioavailability, and inhibiting platelet coagulation and aggregation [31].

3.5. Changes in rat's weight

Body weight development in rats has two stages: advancement to development in which the development rate is tall, and all body parts develop, and post-maturity development in which the development rate is lower than the past arrange. *Rattus norvegicus* is a fairly large member of the rats family. The average weight of these rat reaches 140 to 500 g. Changes in body weight rats for 28 days are shown in Figure 5.

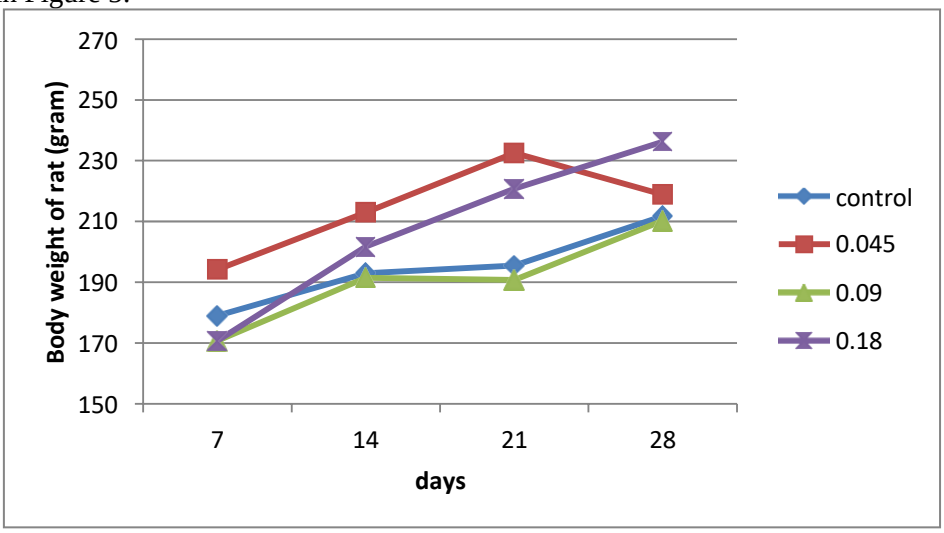

Figure 5. Changes in body weight of rats for 28 days

The results of the weight analysis of white rats for four weeks after being given effervescent tablets can be seen in Fig 5. Administration of synbiotic effervescent tablets on Dose A, Dose B, and Dose C caused an increase in rat body weight. The percentage increase in doses A, B, and C are 12.61%, 23.12%, and 38.36% respectively.

Based on Fig 5, the higher dose, will affect the results of the percentage change in body weight in white rats. In this study, there was no correlation between changes in body weight and increases or decreases in cholesterol, triglycerides, HDL, and LDL levels. In general, the administration of effervescent tablets can affect improving digestive metabolism through prebiotic compounds and probiotics. Likewise, the treatment in this study did not distinguish the type of food. Synbiotic effervescent tablets could help the breakdown of compounds contained in feed without reducing the food intake and body weight of rats.

The results of this study showed changes in the body weight of rats from the beginning of treatment to the end of treatment. This difference in body weight cannot be separated from the food intake in each experimental group. Consumption of a diet rich in carbohydrates and fats will cause an increasein the amount of fat deposited in adipose tissue, especially under the skin and in the abdominal cavity [32]. Any excessive amount of dietary fats and carbohydrates that are not directly used will be storedin adipose tissue as triglycerides. When needed, triglycerides will be hydrolyzed into free fatty acids and glycerol.

4. Conclusion

Treatment using Synbiotic Effervescent tablets at all doses for 28 days can lower total cholesterol, triglycerides, and LDL and increase HDL. The highest cholesterol reduction (49.94%) was found in rats given tablet doses twice the normal dose for 28 days. Based on the highest decrease in cholesterol levels obtained in treatment with the number of doses of effervescent tablets 0.18 grams/head/day (2x the normal dose) with a percentage decrease in cholesterol levels reaching 49.94%. Giving synbiotic effervescent tablets at a dose on day 21 reduced rat cholesterol levels to reach normal limits.

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