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Hypolipidemic Effects of Cake from Butyrylated Arrowroot Starch

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ABSTRACT

The research was conducted to determine hypolipidemic effects of cake from butyrylated arrowroot starch (BAS). The research was conducted using complete randomized design (CRD) consisting of one simple factor: the proportion of cake from BAS. The study was conducted by using 36 Sprague Dawley rats. The mice adapted for 6 days. On 6^{th} day conducted lipid profile analysis and grouping of the mice, so that each group had an average triglyceride level of the same. Rats were divided into 6 groups, each group consisting of 6 tails. Starting on 7^{th} day rats were fed according to the treatment, which consists of 6 levels, which AIN93 standard feed, the feed of the cake from BAS: 0%, 25%, 50%, 75% and 100%. Feed the treatment given for 28 days. The results showed that feeding 100% of the cake from BAS quite effective to reduce the concentration of total cholesterol, LDL cholesterol, triglycerides, and increase HDL cholesterol concentrations Sprague Dawley rats. The largest decrease occurred in the proportion of feeding cake from BAS 100%, respectively at 23.61; 23.02, and 9.71% for cholesterol total, LDL cholesterol and triglyceride levels compared with the standard feeding, while the levels of HDL cholesterol actually increased by 4.23%.

Keywords: *Hypolipidemic effect, resistant starch, butyrylated arrowroot starch, cake*

1. INTRODUCTION

Arteriosclerosis is one of the causes of coronary heart disease, one of disease that currently suffered many people. This disease is caused by the accumulation of lipids and cholesterol in the blood with a high concentration (Finley et al, 2007). One of effective way to reduce the concentration of lipids and cholesterol in the blood are by consuming a lot of foods that contain lots of fiber or resistant starch. Resistant starch is a starch that cannot be degraded by the enzyme α-amylase in the small intestine and into the colon to be used as a substrate for lactic acid bacteria to produce SCFA (Short Chain Fatty Acid), especially acetic acid, propionic acid and acid butyrate. According to Han et al., (2004), that propionate can reduce the accumulation of cholesterol in the blood serum and in rat liver.

Soluble dietary fiber and resistant starch can reduce LDL cholesterol concentrations in normal subjects and those with hyperlipidemic (Romero et al., 1998). Therefore consume foods rich in dietary fiber or modified starch in an amount sufficient can give effect to the body healthy. Other results also show that rats fed a starch containing resistant starch from red beans were known to have serum cholesterol and triacylglycerol concentrations were lower when compared to those fed standard (Han et al. 2004). Similar results were also obtained based on the results of research conducted by Annison, et al., (2003). Based on these results it is known that giving asetilat starch from corn starch to rats causes the starch is not absorbed in the small intestine, but get into the colon and utilized by the micro flora to produce short-chain fatty acids (Short Chain Fatty Acids = SCFA). SCFA produced by the micro flora present in the colon has an important role in the process of decline in concentration of lipids, total cholesterol and LDL cholesterol in mice.

Based on the results of a previous study conducted by Damat et al (2008), by using butyrylated arrowroot starch with a different degree of substitution is known that the use of butyrylated arrowroot starch effective enough to reduce the concentration of lipids, total cholesterol and LDL cholesterol to mice. Besides, it is known that more the degree of substitution, more effective for lowering lipid concentrations, total cholesterol and LDL cholesterol to mice. However, the question is whether the butyrylated arrowroot starches been made cake can also provide physiological effects similar to the physiological effects of butyrylated arrowroot starch. Therefore, it is interesting to do a study to know the lipidemic effect of cake from butyrylated arrowroot starch. The research was conducted with aim to know the hypolipidemic effect of the cake from butyrylated arrowroot starch to Sprague Dawley rats.

2. MATERIALS AND METHODS

2.1 Material

The main material for this study is butyrylated arrowroot starch obtained from modified by esterification way between arrowroot starch with butyric anhydride. Other materials are materials for making cake. To test the hypolipidemic effects in vivo used mice. A total of 36 (thirty six) male Sprague Dawley rats 3 months of age with body weight of 250-300 grams obtained UPTP (Technical Services Research Unit), Gadjah Mada University, Yogyakarta.

Before test the physiological effects were done, it was prepared feed and feed treatment standards first. The feed is formulated according to standard AIN93 (American Institute of Nutrition), (Reeves et al., 1993). Rats feed ingredients that needed include cornstarch, soy oil, sucrose, CMC and whey protein concentrate. Other materials are alloxan, AIN93 mineral mixture MX, AIN93 VX vitamin mix, L-Sistine Alphacel, non-nutritive bulk, tert-Bytylhydroquinone, choline bitatrate and vitamin E. For the analysis of lipid profiles required several types of materials include amylase, amyloglukosidase, pululanase (Merck), acetone, kits for the analysis of lipid profile (triglycerides, cholesterol, LDL and HDL) and a kit for analysis of blood serum glucose (DiaSys Dianostic Systems International).

2.2 Research Method

The research was conducted in three phases. The first stage of the preparation of butyrylated arrowroot starch with a degree of substitution (DS) 0.187 as developed by Damat (2008). The second phase cake manufacture with the proportion from arrowroot starch to flour butyrate as big as 0, 25, 50, 75 and 100%. The third phase, testing cake hypolipidemic effect from butyrylated arrowroot starch on Sprague Dawley male rats, weighing 250-300 grams. Before fed treatment, rats adapted for 6 (six) days. Feeding was done by ad libitum way. First feeding amount as much as 15 grams / head / day. The rest of the feed every day weighed. On 6^{th} mice performed grouping. Rats were divided into 6 groups, and each group consisted of 6 rats, so that total there are 36 tails. Furthermore, mice were fed according to the treatment and one group was fed a standard AIN93. Rats maintained individually (one tail / cage) for 6 days of adaptation and 28 days of treatment.

Before feeding in rats, cake from butyrylated arrowroot starch analyzed its chemical composition that includes analysis of water content, fat content, ash content, protein content and resistant starch content. Analysis of resistant starch was done by using the method of Englyst and Cummings (1988). The analysis of total cholesterol content, LDL cholesterol, HDL cholesterol, and total triglycerides in rats performed at 6^{th} day, 13, 20, 27 and 34. Analysis of total cholesterol performed by CHOD-PAP method (Richmond, 1973), LDL cholesterol by CHOP-PAP method (Wieland and Siedal, 1983), analysis of HDL cholesterol by CHOP-PAP method (Eckal et al., 1977), and the total triglycerides by GPO-PAP method (Mc Gowan et al., 1983).

3. RESULTS AND DISCUSSION

3.1 Chemical Materials Composition

Before being used for the manufacture of feed diet rats, proximate analysis and analysis of resistant starch were done on the cake composition made with 100% wheat flour composition (C0), 25% butyrylated arrowroot starch, 75% wheat flour (C1), 50% butyrylated arrowroot starch, 25 % wheat flour (C2), 75% arrowroot starch butyrylated, 25% wheat flour (C3) and 100% arrowroot starch butyrylated (C4). The results of this analysis are used as the basis for determining the composition of feed mixtures for research with mice Sprague Dawley (SD).

Table 1: The chemical composition of arrowroot starch and flour (%).

Kind of materials	Water content (%)	Ash content (%)	Protein content (%)	Lipid content (%)	Carbohydrate content (%)	Resistant Starch (%)
BAS	11,98	3,17	3,69	0,80	80,36	13,94
Cake from 100% wheat (C_0)	29,55	2,10	8,74	3,29	56,32	1,56
Cake from 25% BAS, 75% wheat flour (C1)	28,93	2,40	8,12	3,85	56,70	2,43
Cake from 50% BAS, 50% wheat flour (C_2)	27,54	2,53	8,07	3,92	57,94	4,78
Cake from 75% BAS, 25% wheat flour (C_3)	27,19	2,55	7,99	4,24	58,03	6,31

BAS= butyrylated arrowroot starch

According to the Table 1, it was found that the cake was made from 100% wheat flour (C0) has a 1.56% resistant starch concentration and then rose to 8.61% on the cake that was made from 100% butyrylated arrowroot starch and flour 0% (C4). The increase was due to the increasing proportion of butyrylated arrowroot starch on the cake. From Table 1 it was known that the concentration of resistant starch on the cake bigger along with the increasing proportion of butyrylated arrowroot starch. Resistant starch is a starch that cannot be digested in the small intestine by amylolytic enzymes and will go into the colon to be substrates for micro and produce SCFA (Short Chain Fatty Acids) (Higgins, et al., 2004). Proximate analysis results are then used as the basis for determining the composition of the diet feeding mice. Feeding mice diet formulated on a standard AIN 93 formula feed.

3.2 Total Cholesterol

Total cholesterol concentrations after giving of dietary adaptation, and after the diet treatment can be seen in Figure 1. At the beginning of the maintenance (day-1),

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the average total cholesterol concentration as big as of 108.71 mg / dl and after giving of adaptation diet for 6 days, the average cholesterol concentrations rose to 110.23 mg / dl, or increase about 1.40 %.

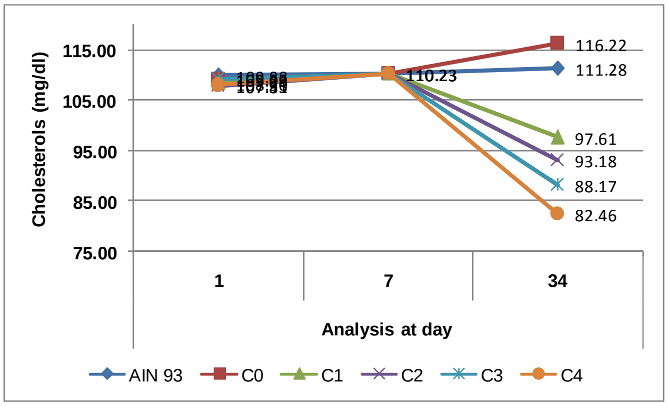

Fig 1: Concentrations of total cholesterol mice throughout the study

Dietary administration cake from butyrylated arrowroot starch effective enough to reduce total cholesterol concentration. Total cholesterol concentration at day 34 in mice that were fed a standard AIN93 of 116 mg / dl, and fed cake diet from flour (C0) of 111.28 mg / dl, while those fed cake diet from butyrylated arrowroot starch as much as 97, 61; 93.18; 88.17 and 82.46 mg / dl, respectively cake diet of butyrylated arrowroot starch with proportion butyrylated 25, 50, 75 and 100%. Based on the analysis, it was found that the greatest reduction in total cholesterol occurred on mice fed cake diet of 100% butyrylated arrowroot starch. Total cholesterol reduction reaches 23.61%.

Based on Figure 1 above, it appears that higher the proportion of butyrylated arrowroot starch on the cake, greater the reduction percentage in total cholesterol concentration in mice Sprague Dawley. This is due to the greater proportion of butyrylated arrowroot starch on the cake, the greater the percentage of resistant starch on the cake (Table 1) which are viscous. Viscous properties believed to increase layer thickness between the food and the brush-border surface in the small intestine, preventing nutrients absorption (Marsono, 2001).

Hypocholesterolemic effect of cake-butyrylated arrowroot starch due to its ability to inhibit the absorption of bile acids and steroid compounds on the small intestine so that bile acids and total steroids are excreted increased. Han et al. (2004) reported that the effect of a decrease in total cholesterol concentration in the blood plasma by resistant starch due to the increase of steroid excreted with feces. The results of recent studies indicate that administration of dietary resistant starch from Kintoki can significantly increase the excretion of bile acids compared with the cellulose diet fed. This is a typical characteristic of the resistant starch that has the ability to bind bile acids, increases the viscosity of intestinal contents and reduces the absorption of bile acids from the intestine. It was also known that cholesterol 7α-hydroxylase mRNA on mice fed resistant starch diet is higher compared with control (Kishida et al. (2002). According to Finley (2007), the effect of cholesterol reduction by resistant starch was highly dependent on the LDL receptor, 7α-hydroxylase mRNA cholesterol level, steroid excretion with feces and butyric acid production speed. Based on these results, it can be concluded that resistant starch has a positive influence on lipid metabolism on the liver.

3.3 LDL Cholesterol

An LDL cholesterol concentration after administration of dietary adaptation and after administration of dietary treatment is shown in Figure 2. At the beginning of maintenance (day-1) the average concentration of LDL cholesterol totaling 51.76 mg / dl and after the adaptation diet for 6 days, the average concentration of LDL cholesterol was increased to 52.30 mg / dl or an increase of about 1.04%.

Cake diet administration from butyrylated arrowroot starch was known effective enough to reduce total LDL cholesterol concentration. LDL cholesterol concentration at day 34 on mice that were fed AIN93 standard totaling 51.20 mg / dl, and fed cake diet from flour (C0) totaling 52.00 mg / dl, while those fed cake diet butyrylated arrowroot starch at 46.54; 42.23; 41.80 and 40.26 mg / dl, respectively cake diet of arrowroot starch with proportional butyrylated 25, 50, 75 and 100%. Based on the analysis it was found that the greatest reduction in LDL cholesterol occurred in mice fed a diet of 100% butyrylated cake arrowroot starch. Decrease in LDL cholesterol reaches 23.02%.

Based on Figure 2, it appears that the higher the proportion of butyrylated arrowroot starches on the cake, the greater the percentage reduction in LDL cholesterol concentration in mice Sprague Dawley. This is due to the greater proportion of butyrylated arrowroot starch on the cake, the greater the percentage of resistant starch on the cake (Table 1). Decrease on the concentration of LDL cholesterol in rats fed cake diet arrowroot starch-resistant caused starch butyrate which contained on the cake has the

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ability to bind bile acids, increase the intestinal contents viscosity that can inhibit absorption various types of macronutrient, including lipids, and reduced bile acid absorption from the small intestine via the enterohepatic circulation.

Result was similar to the research result conducted by Han et al., (2004), which states that rat fed resistant starch significantly from Kintoki known has total cholesterol concentration was lower when compared to the control. The decrease on total cholesterol was also followed by decrease on the concentration of cholesterol VLDL, IDL, LDL and HDL cholesterol. Reduced concentration of LDL cholesterol is an important factor that can lead to decreased total cholesterol concentration. The result of other study conducted Finley (2007) was known that rats fed resistant starch has hepatic LDL receptor mRNA were higher when compared to the controls, so as to increase the activity of LDL receptor and SR-B1 in the liver. SR-BI (Scavenger receptor class B, type I) is a membrane protein present on the cells or liver tissue or adrenal. As the LDL receptor, SR-B1 functions to capture cholesterol esters from HDL in the liver. This process can encourage cholesterol transfer from peripheral tissues to the liver and then excreted through the feces. Cholesterol transfer is called reverse cholesterol transport and it is a mechanism to prevent atherosclerosis, a condition that can lead to stroke.

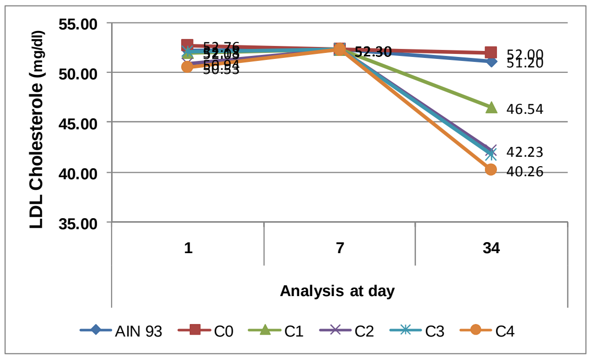

Fig 2: Concentrations of LDL cholesterol mice throughout the study

3.4 HDL Cholesterol

HDL cholesterol concentration after administration of dietary adaptation and after the diet treatment can be seen in Figure 3. At the start of the maintenance (day-1) the average concentration of HDL cholesterol by 52.52 mg / dl and after administration adaptation diet for 6 days, the average concentration of HDL cholesterol rose to 52.80 mg / dl or an increase of about 2, 37%.

Dietary administration cake from butyrylated arrowroot starch effective enough to increase HDL cholesterol concentration. HDL cholesterol concentration on day 34 on rat which was fed a standard AIN93 of 41.20 mg / dl, and fed cake diet of flour (C0) totaling 42.00 mg / dl, while those fed cake diet butyrylated arrowroot starch at 43.21; 44.20; 44.50 and 44.61 mg / dl, respectively cake diet of arrowroot starch butyrylated with proportion 25, 50, 75 and 100%. Based on the analysis, it was found that the greatest increase in HDL cholesterol occurred in rat which fed cake diet of 100% butyrylated arrowroot starch. The increase on HDL cholesterol reached 4.23%.

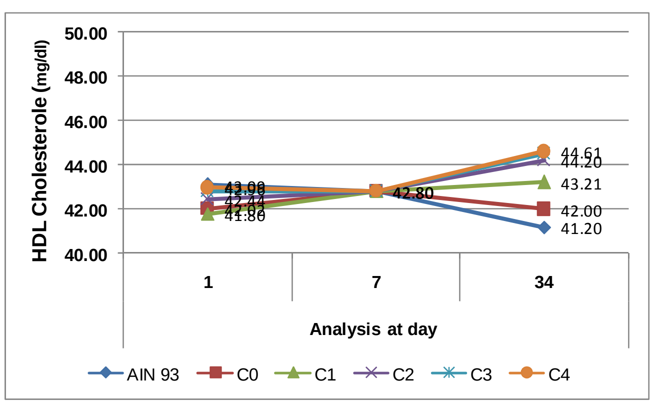

Fig 3: Concentrations of HDL cholesterol mice throughout the study

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Based on Figure 3 above, it appears that higher the proportion of butyrylated arrowroot starch on the cake, greater the percentage increase in HDL cholesterol concentration on Sprague Dawley rat. This is due to greater proportion of butyrylated arrowroot starch on the cake, greater the percentage of resistant starch on the cake (Table 1). These results contrast with results of previous studies which showed that administration of dietary resistant starch on rat was known to reduce the concentration of HDL cholesterol on rat. Han et al. (2004) stated that rat fed resistant starch from Kintoki significantly has concentrations of total cholesterol, HDL cholesterol; VLDL, IDL, and LDL cholesterol were lower when compared to the control. Decrease in HDL cholesterol concentrations in rats fed RS due to accelerated spending HDL through the hepatic HDL receptor. This is because RS can increase the expression of SR-B1 mRNA and decrease concentrations of HDL cholesterol.

3.5 Triglyceride

Triglyceride concentrations after administration of dietary adaptation and after administration of dietary treatment are shown in Figure 4. At the beginning of maintenance (day-1,) the average concentration of triglycerides by 72.17 mg / dl and after diet adaptation for 6 days, the average concentration of triglycerides rose to 73.04 mg / dl or an increase approximately 1.21%.

Dietary administration cake from butyrylated arrowroot starch effective enough to reduce triglycerides concentration on Sprague Dawley. Triglyceride concentration on 34th day on rat which was fed AIN93 standard totaling 74.10 mg / dl, and fed cake diet from flour (C0) totaling 74.34 mg / dl, while those fed cake diet from butyrylated arrowroot starch for 72.10; 68.21; 66.92 and 65.30 mg / dl, respectively cake diet of arrowroot starch butyrylated with proportion of 25, 50, 75 and 100%. Based on analysis, it was found that greatest reduction on triglycerides occurred on rat fed cake diet from 100% butyrylated arrowroot starch. The decrease triglyceride levels reached 9.71%.

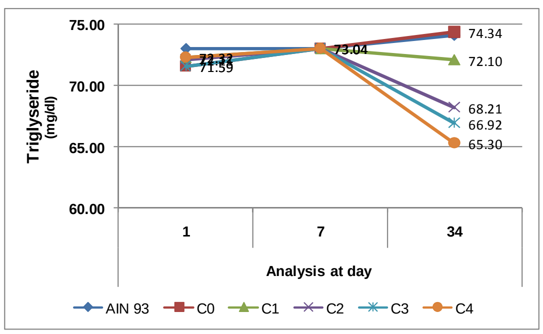

Fig 4: Concentrations of triglyseide mice throughout the study

Based on Figure 4 above, it appears that higher the proportion of butyrylated arrowroot starch on the cake, greater percentage decrease the triglycerides concentration on Sprague Dawley rat. This is due to the greater proportion of butyrylated arrowroot starch on the cake, greater the percentage of resistant starch on the cake (Table 1). This result was similar to the research result conducted by Higgins et al., (2004). Based on these results, it was known that the replacement of as much as 5.4% carbohydrates with resistant starch significantly increased postprandial lipid oxidation, so that in the long-term will reduce the accumulation of fat on experimental animals (Higgins, et al., 2004).

In addition to the factor above, decrease triglyceride concentration also caused by decreased adipose cell size and enzyme activity lipogenic. This is appropriate with the research result conducted Behall, at al., (2006), and which states that dietary resistant starch administration on rat can cause decrease on adipose cell size. The results of other studies conducted by Kabir et al., (1998) were known that the addition of resistant starch on foods can cause strong physical association between resistant starch and lipids, so that decreasing absorption. Thereby enhancing lipid oxidation, so that triglycerides concentration will decrease.

In addition, it was known that consumption of resistant starch can decrease postprandial lipogenesis on rat adipose tissue, so that in the long-term can lose weight. The results of other studies by using pigs as experimental animals also showed that consumption of resistant starch could significantly decrease lipogenesis on adipose tissue, increasing amount of fat lost from mouse body, especially fat on the abdominal part. The researchers suspect that the response is an expression of resistant starch to the glucagon-like peptide (GLP-1). This shows that by consuming RS can enhance the hormones signal, so that resistant starch can be used to prevent obesity. It is generally known that the resistant starch administration on animals could lead to decreased cell size adipocytes (fat cells) (Al-Tamimi, 2007).

4. CONCLUSION

Cake made from butyrylated arrowroot starch effective enough to reduce the concentration of total cholesterol, LDL cholesterol, triglycerides and increase HDL cholesterol concentrations on Sprague Dawley rats. Concentration decrease on total cholesterol, LDL cholesterol, and greatest triglycerides on rats fed 100% cake diet of arrowroot starch butyrate, respectively reached 23.61%, 23.02% and 9.71%, while the highest increase on HDL cholesterol achieved 4.23% also occurred in the same treatment. Based on these results, it could be concluded that the greater proportion of butyrylated arrowroot starch on the cake, the more effective for lower concentration of total cholesterol, LDL cholesterol, triglycerides, and more effective way to increase HDL cholesterol in Sprague Dawley rat.

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