

The Effect Of Application Of Cavendish Jepara 30 Banana Pseudostem Flour On Production Of Short-Chain Fatty Acids And Cholesterol In Caecum Digesta Of Hypercholesterolemic Mice

Yuliatmoko Welli, Murdiati Agnes, Pranoto Yudi, Marsono Yustinus

Abstract: Cavendish Jepara 30 banana pseudostem flour (EBP flour) was reportedly rich in bioactive components. The objective of study was to determine the effect of EBP flour and canna-starch-EBP based food bar on the production of short-chain fatty acids and cholesterol in the caecum digesta reduction in terms of lowering blood cholesterol. One group of normal mice consisted of 5 mice were given standard food diet. Five groups of hypercholesterolemic mice, each 5 mice, fed with a standard diet and EBP flour. The analysis on caecum digesta consisted of water content, weight of digesta caecum, pH, levels of short-chain fatty acids, and cholesterol levels. Dietary interventions of canna starch-EBP based food bar produced the highest concentration of propionic acid of $38.1 \pm 15.3\%$ followed by blanched EBP flour $18.1 \pm 9.1\%$. However, the intervention diet of blanched EBP flour showed a higher number of cholesterol of caecum digesta compared to the canna-starch-EBP based food bar and natural EBP flour diet, 83.9 ± 2.1 mg GAE/100 g, 79.1 ± 1.2 mg GAE/100 g, and 53.3 ± 1.4 mg GAE/100 g. To conclude, blanched EBP flour and canna starch-EBP based food bar increased the concentration of propionic acid and cholesterol excretion on caecum digesta so that both could benefit for health.

Key Words : Caecum, cavendish Jepara 30, cholesterol, digesta, food bar, propionate, resistant starch.

1. INTRODUCTION

NOWADAYS lifestyle of society has triggered the emergence of various diseases that threaten the world's population such as hypertension, diabetes, and dyslipidemia [1]. Dyslipidemia is a blood lipid profile abnormalities such as increased total cholesterol, LDL, triglycerides, and reduced HDL cholesterol [2, 3]. Dyslipidemia is a major risk factor for coronary heart disease [4, 5]. Dyslipidemia should be treated because it could trigger more severe diseases like heart coronary disease. On the other hand, bioactive component such as dietary fiber (DF) had been reported to be hypocholesterolemic [6, 7]. Resistant starch (RS) which had properties such as water-soluble fiber was also reported to be hypocholesterolemic [7, 8]. The first proposed solution in dealing with dyslipidemia was still using cholesterol-lowering drugs such as simvastatin and cholestyramine [9]. The use of drugs in dealing with dyslipidemia in a long time could cause adverse side effects for patients, such as myopathy and rhabdomyolysis [9, 10]. Another alternative in dealing with dyslipidemia condition was to consume a variety of natural materials such as DF and RS contained in food or various processed products enriched with these materials [11].

Treatment of dyslipidemia in this method was safer and economical because it used natural ingredients and less expensive as the availability of DF and RS components not need to purchase expensive ingredients, but only needed plants, fruits, and processed products locally. EBP flour was reportedly rich in bioactive components such as DF, RS, and antioxidants [12]. This flour also could increase the content of dietary fiber, resistant starch and antioxidant canna-starch based food bar which was substituted with Jepara 30 banana pseudostem flour (canna starch-EBP food bar) [13]. Bioactive components such as DF and RS in flour and a food bar had the potential used for health, especially to lower the cholesterol. Capability of DF and RS component in reducing dyslipidemia condition was affected by several factors such as the origin of the materials, processing methods, and others. Besides, many studies made use of short-chain fatty acid (SCFA) and caecum digesta cholesterol to determine the ability of dietary fiber and resistant starch in improving the condition of dyslipidemia. Therefore, the capability of DF and RS component on EBP flour to reduce cholesterol was urgent to investigate. In contrast, there was no updated study that revealed about the capability of the DF and RS on EBP flour and canna-starch-EBP based food bar in improving the condition of dyslipidemia. This study aimed to determine the effect of EBP flour and canna-starch-EBP based food bar to the properties of the caecum digesta and cholesterol of hypercholesterolemic mice and the possible mechanism.

2 MATERIAL AND METHODS

2.1 Materials

The raw material used was natural Cavendish Jepara 30 EBP flour (A1), the EBP flour processed without treatment; blanched EBP flour (A2), EBP flour processed by blanching treatment at 100 °C for 10 mins [12]; and canna-starch-EBP

- Yuliatmoko Welli is currently pursuing PhD degree program in food science at the Department of Food Technology and Agricultural Products, Faculty of Agricultural Technology, Gadjah Mada University, Jl. Flora No. 1, Bulaksumur 55281, Yogyakarta, Indonesia. E-mail: welli@mail.ugm.ac.id
- Co-Author names (Murdiati Agnes, Pranoto Yudi, and Marsono Yustinus) are lecturers in the food science doctoral program at the Department of Food Technology and Agricultural Products, Faculty of Agricultural Technology, Gadjah Mada University, Jl. Flora No. 1, Bulaksumur 55281, Yogyakarta, Indonesia

based food bar (A3), a food bar which was processed using canna starch that substituted with blanched EBP flour with ratio of canna starch: blanched EBP flour 85:15 [13].

2.2 Handling of test animals

Sprague Dawley male mice, 2 months old, 150-200 g body weight as much as 30 mice were adapted, nurtured by drinking and eating with standard feed in ad libitum for 4 days. Furthermore, mice were weighed and their blood was collected for lipid profile test as the early treatment. Mice were grouped into two: normal (5 mice) were fed with a standard feed diet AIN 93 M [14], and hypercholesterolemic mice (25 mice) were fed with diet containing dietary cholesterol 10 g.1000⁻¹ g and Na cholate 2.5.1.000⁻¹ g of weight of feed during 7 days [15]. To determine the condition of cholesterol was achieved the criteria; the lipid profile analysis was done at blood cholesterol levels > 200 mg.dl⁻¹ [16]. Subsequently, mice were divided into 6 treatments: normal mice fed with the

standard AIN 93 M (normal control/B1), group of hypercholesterolemic mice were fed with standard AIN 93 M without treatment (negative control/B2), group of hypercholesterolemic mice were fed with natural Cavendish Jepara 30 EBP flour (B3), group hypercholesterolemic mice were fed with blanched Cavendish Jepara 30 EBP flour, (B4), hypercholesterolemic mice were fed with canna starch-based food bar which was substituted with flour Cavendish Jepara 30 EBP (B5), and a group of hypercholesterolemic mice were fed standard AIN 93 M with medication of simvastatin 3ml.200⁻¹ g (positive control/B6) [17]. Intervention feed for 4 weeks by following diet (Table. 1) and enough water. On day 31, mice were anesthetized and dissected to take caecum digesta for further analysis. This research has obtained ethical client from the faculty of medicine at Gadjah Mada University as stated in Ref: KE / FK / 1076 / EC / 2018.

TABLE 1
COMPOSITION OF STANDARD FEED AND TREATMENT (G.KG⁻¹)*

Composition	Diet					
	B1	B2	B3	B4	B5	B6
Protein: casein	140.0	140.0	111.1	110.8	66.7	140.0
Corn flour	620.7	620.7	611.8	702.6	156.5	620.7
Sucrose	100.0	100.0	100.0	100.0	100.0	100.0
Soybean oil	40.0	40.0	39.1	39.0		40.0
CMC	50.0	50.0				50.0
A1			108.4			
A2				114.1		
A3					659.6	
Mineral mix	35.0	35.0	1.2	5.3	17.3	35.0
Vitamin mix	10.0	10.0	10.0	10.0	10.0	10.0
L-cysteine	1.8	1.8	1.8	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5
Total energy [J]	15.921.6	15.921.6	16.407.2	18.081.9	17.617.0	15.921.6
Feed weight [g]	1.000.0	1.000.0	1.087.0	1.093.0	1.670.0	1.000.0

B1 – normal fed with standard AIN 93 M, B2 – hypercholesterolemia fed with standard AIN 93 M without treatment, B3 – hypercholesterolemia fed with natural Cavendish Jepara 30 EBP flour, B4 – hypercholesterolemia fed with blanched Cavendish Jepara 30 EBP flour, B5 – hypercholesterolemia fed with canna starch-based food bar which was substituted with flour Cavendish Jepara 30 EBP, and B6 – hypercholesterolemia fed with standard AIN 93 M with medication of simvastatin 3ml.200-1 g; A1 – natural Cavendish Jepara 30 EBP flour, A2 – blanched EBP flour, A3 – canna-starch-EBP based food bar, * – [13]

2.3 Analysis of water content and weight of digesta

Analysis of water content digesta was performed as in [18]. Digesta from each mouse was isolated and the entire contents of digesta was weighed, while the water content of digesta were analyzed by gravimetric method. Digesta samples diluted by using distilled water with a certain amount included in an oven at a temperature of 100-105 °C for 8 h. The weighing was done until it reached a constant weight.

2.4 Short Chain Fatty Acid analysis and pH

Short Chain Fatty Acid (SCFA) analyzes of mice digesta were conducted by following procedures [19]. Mice dissected, colon and digesta taken out. Digesta was centrifuged at a speed of 21952 g for 15 min. The supernatant was taken for analysis by gas chromatography (GC). Supernatant injected into the GC

column with the following conditions: column GP 1200/1% HPP30 on chromosorb waw, 2-m long column, column temperature was set at 130 °C, injector temperature of 230 °C detector, nitrogen carrier gas with a pressure of 1.25 kg. cm⁻², machine GC Shimadzu GC series 8. Meanwhile, pH analysis conducted following the procedures [20]. Digesta pH was observed by suspending free ion aquabidest in sterile deionized (1:10 w/v) as soon as possible after sampling. Subsequently, it was mixed and vortexed. pH was measured with pH meter by connecting the electrodes with the fitting, then inserted in the sample that had been diluted with distilled water. The value measured on a pH meter was pH of the caecum fluid.

2.5 Determination of SFCA Molar Ratio

SCFA molar ratio (%) was determined by calculating the ratio of the percent of each of propionic acid, acetic acid, and butyric acid. Data of third molar ratio of the acid obtained from the SCFA concentration data.

2.6 Analysis of Cholesterol Digesta

Cholesterol digesta was analyzed by the following method [21]. A sample of 1 g digesta caecum digesta was put in a test tube, then mixed with 10 ml acetone-alcohol solution, then heated in a water bath filled with boiling water, cooled at room temperature. Then the filtrate was taken and centrifuged for 15 min at 700 g. The supernatant is evaporated to dryness in waterbath at 100°C, then cooled at room temperature, then dissolved with 3 ml chloroform solvent and 3 ml concentrated anhydride-sulfuric acid acetic acid solution, homogeneous and placed in a dark room for 5 min until bluish green. A blank solution is made in the same way. Samples and blanks were measured for absorbance at a wavelength of 680 nm. The level of digesta caecum is calculated by comparing the absorbance of the sample with the absorbance of standard cholesterol.

2.7 Determination Experimental Design

Digesta water content, digesta weight, digesta pH, SCFA digesta level, and digesta cholesterol level were measured 5 times. Data were processed using one-way analysis of variance followed by the Least Significant Difference conducted in SPSS version 20.0 [22].

3 RESULTS AND DISCUSSION

3.1 The water content and weight of digesta

The results of the analysis of water content of caecum digesta varied among treatments as presented in Fig. 1. Dietary interventions of natural EBP flour (B3), blanched EBP flour (B4) and canna-starch-EBP-based food bar (B5) could increase the water content of digesta with the subsequent order of

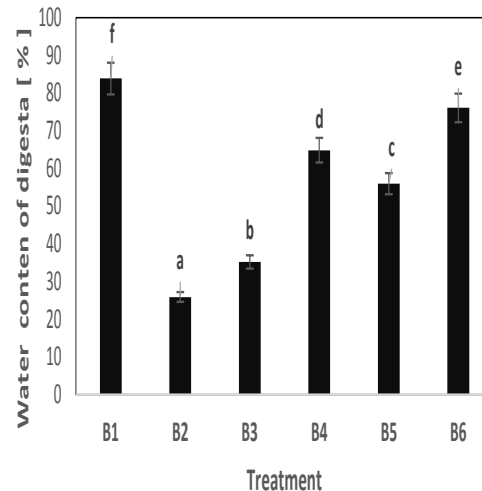


Fig. 1. The water content on digesta of mice in each treatment. Normal fed with standard AIN 93 M (B1), hypercholesterolemia fed with standard AIN 93 M without treatment (B2), hypercholesterolemia fed with natural Cavendish Jepara 30 EBP flour (B3), hypercholesterolemia fed with blanched Cavendish Jepara 30 EBP flour (B4), hypercholesterolemia fed with canna starch-based food bar which was substituted with flour Cavendish Jepara 30 EBP (B5), and hypercholesterolemia fed with standard AIN 93 M with medication of simvastatin 3ml.200⁻¹ g (B6). The averaged values followed by different letters was significantly different ($p < 0.05$).

increased level: B4 > B5 > B3. This was demonstrated by the high levels of water treatment of B4, B5 and B3, compared to treatment B2 (negative control). However, their increased level in water levels were still under the level of treatment B1 (normal control) and B6 treatment (positive control). Increased levels of water content were thought to originate from the DF and RS contained by feeding diets A2, A3 and A1. DF and RS components in diet could increase the water content of digesta [23, 24]. DF even could improve the ability of diet to absorb 10-fold water when compared with non-DF diet [25]. It was due to the properties of dietary fiber that had a high water binding capacity (water holding capacity/WHC). High levels of water treatment digesta, B1 and B6, allegedly due to WHC ability of dietary fiber that was both higher. WHC ability of dietary fiber was affected by the chemical structure of the fibers, the species, and the anatomy of the material [26] to [24] as well as particle size and pH [8, 27]. The results of the analysis of the caecum digesta weight of mice varied among treatments as presented in Fig. 2. Dietary interventions of banana pseudostem flour and canna starch-EBP based food bar could increase the weight of the caecum digesta of mice with the order of increase: B5 > B4 > B3. This was demonstrated by the high weight of the caecum digesta on treatment B5, B4, B3 and B2 when compared to other treatments. The intervention

diet B5 showed increased weight of the caecum digesta and significantly different than the standard feed diet ($p < 0.05$). In normal control treatment, the digesta weight was not significantly different compared to standard diet on the positive control treatment. The increase in weight of the caecum digesta was thought to originate from the DF and RS contained on A3, A2 and A1 diets. DF and RS components in diet could increase the weight of the caecum digesta [23, 24]. Increasing the caecum digesta was closely related to the high water content of digesta and the increase in the number of bacterial mass resulting from the fermentation of dietary fiber [28].

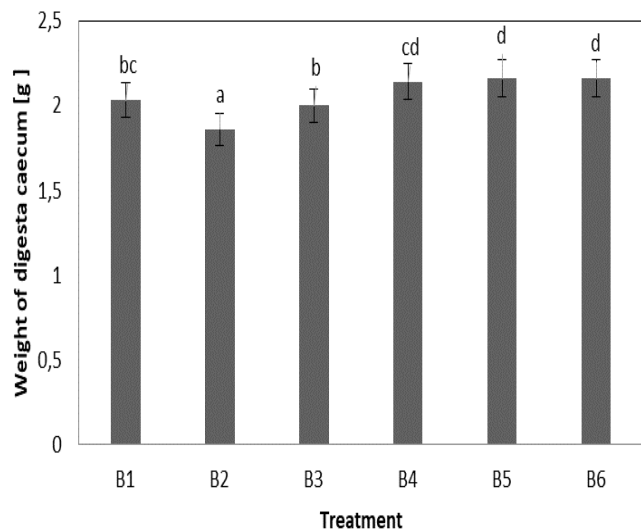


Fig. 2. The weight of digesta caecum cholesterol of mice in each treatment. Normal fed with standard AIN 93 M (B1), hypercholesterolemia fed with standard AIN 93 M without treatment (B2), hypercholesterolemia fed with natural Cavendish Jepara 30 EBP flour EBP flour (B3), hypercholesterolemia fed with blanched Cavendish Jepara 30 EBP flour (B4), hypercholesterolemia fed with canna starch-based food bar which was substituted with flour Cavendish Jepara 30 EBP (B5), and hypercholesterolemia fed with standard AIN 93 M with medication of simvastatin $3\text{ml} \cdot 200^{-1} \text{g}$ (B6). The averaged values followed by different letters was significantly different ($p < 0.05$).

3.2 Short Chain Fatty Acid and pH

The analysis result of the concentration of pH of total SCFA, acetic, propionic, and butyric of digesta varied among treatments as presented in Fig. 3. The dietary intervention of B4 and B5 could improve the concentration of total SCFA, acetic, propionic, and butyric with the order of increased level: $B5 > B4$. Treatment B5 show increased concentrations of acetate, propionate, butyrate and total SCFA that was higher and significantly different when compared to the negative control, normal control and positive control. Treatment B4 diet showed concentrations of total SCFA, acetic, propionic, and butyric was higher than the standard feed diet in the treatment of negative control, normal, and positive but not significantly different from normal and positive control. However, a different response shown by diet feed flour EBP naturally given to the treatment A1 shows that dietary intervention feed produces acetic, propionic, butyric, and total SCFA higher than the

treatment of the negative control and did not differ significantly, but still lower and significantly different when compared to normal and positive control. The high concentration of acetic, propionic, butyric, and total SCFA in the diet group B5 and B4 allegedly occurred because of DF and RS contained on A3 and A2 diets. DF and RS components in food could increase the concentration of acetic acid, propionic, and butyric [3, 23, 24]. In addition, the high concentrations of acetic, propionic, butyric, and total SCFA diet on group B5 probably derived from the diverse sources of DF on this feed which were soluble and RS were derived from A2 flour and RS derived from canna starch. A different source of dietary fiber would affect commodity microbes and production of short chain fatty acids [29].

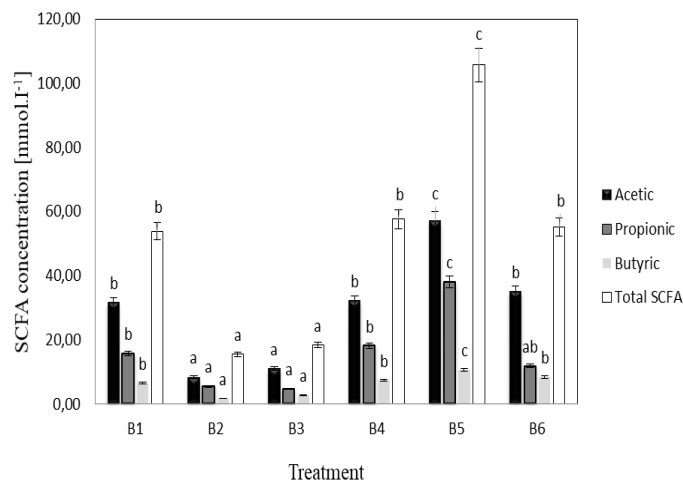


Fig. 3. The concentration of SCFA on digesta of mice in each treatment. Normal fed with standard AIN 93 M (B1), hypercholesterolemia fed with standard AIN 93 M without treatment (B2), hypercholesterolemia fed with natural Cavendish Jepara 30 EBP flour EBP flour (B3), hypercholesterolemia fed with blanched Cavendish Jepara 30 EBP flour (B4), hypercholesterolemia fed with canna starch-based food bar which was substituted with flour Cavendish Jepara 30 EBP (B5), and hypercholesterolemia fed with standard AIN 93 M with medication of simvastatin $3\text{ml} \cdot 200^{-1} \text{g}$ (B6). The averaged values followed by different letters was significantly different ($p < 0.05$). The results of the analysis of SCFA molar ratio varied among treatments as presented in Fig. 4. The dietary intervention of B4 and B5 showed molar ratios with a high propionic acid order: $B5 > B4$. The molar ratio of propionate acid on B5 and B4 was higher than the negative, normal, positive control diet. The molar ratio of propionic acid for both diets was significantly different from the standard feed on the positive control treatment. This condition was allegedly caused by a different source of dietary fiber. Dietary fiber might affect the amount and type of SCFA produced in the colon [23, 29], reported propionic acid could lower blood cholesterol [30]. Thus, the canna starch-EBP based food bar and blanched EBP flour diet were beneficial to health because they could improve blood lipid profile by lowering cholesterol. As seen in Fig. 4, treatment B4 indicated the molar ratio of butyric acid was quite high and did not differ from the standard feed diet in normal and positive control treatment. This indicated that diet could also maintain colonic

health as reported that butyric acid could prevent colon cancer. The results of the analysis of pH of digesta varied among treatments as presented in Fig. 5. The dietary intervention of B4 and B5 could lower the pH of digesta of mice by decreasing order levels: B4 > B5. Blanched EBP flour diet on B4 treatment showed the lowest pH but was not different than standard feed diet in normal and positive control treatment. The low pH of digesta for both groups of mice showed that DF and RS contained in A2 and A3 diet could be fermented in the large intestine (colon) and produced acidic SCFA thus lowering the pH of the caecum digesta. However, the interesting phenomenon shown by B5 feed diet that produced the highest total SCFA but also showed a higher pH than B1, B4 and B6 diet.

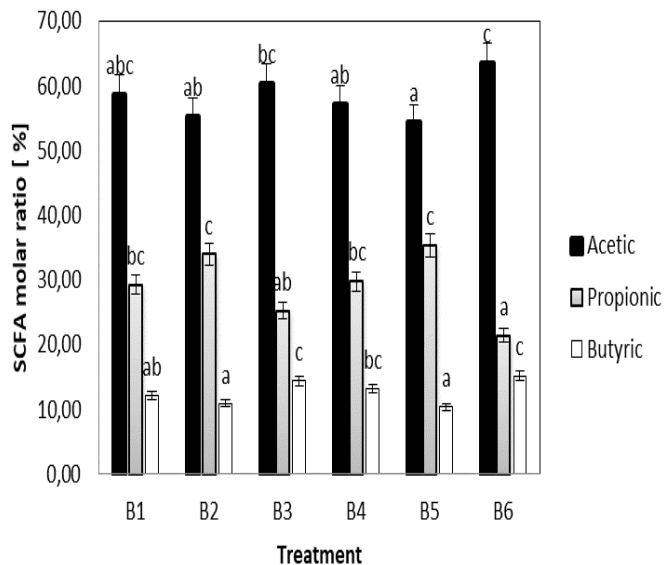


Fig. 4. The molar ratio of SCFA on digesta of mice in each treatment. Normal fed with standard AIN 93 M (B1), hypercholesterolemia fed with standard AIN 93 M without treatment (B2), hypercholesterolemia fed with natural Cavendish Jepara 30 EBP flour EBP flour (B3), hypercholesterolemia fed with blanched Cavendish Jepara 30 EBP flour (B4), hypercholesterolemia fed with canna starch-based food bar which was substituted with flour Cavendish Jepara 30 EBP (B5), and hypercholesterolemia fed with standard AIN 93 M with medication of simvastatin 3ml.200⁻¹ g (B6). The averaged values followed by different letters was significantly different (p <0.05).

3.3 Cholesterol caecum digesta

The results of the analysis of the cholesterol in caecum digesta varied among groups presented on Fig. 6. Dietary interventions of EBP flour and canna starch-EBP based food bar increased the cholesterol in caecum digesta with sequence of increase level: B4 > B5 > B1. This was demonstrated by the high caecum digesta of B4, B5, and B3 treatment when compared with HPS treatment (negative control). However, elevated cholesterol levels in caecum digesta were still under normal and positive control treatment, but significantly different. The presence of the cholesterol in caecum digesta of EBP flour and canna starch-EBP based food bar was probably derived from the activity of soluble RS and DF component containing on diets. Cholesterol in caecum

digesta reportedly came from the secretion of bile and cholesterol which was bound by a DF and RS [32-34]. DF was soluble and able to bind primary and secondary bile acids (sterol or feces cholesterol or coprostanol) and threw it away or excreted simultaneously through the faces so that it could reduce cholesterol levels in the blood as the raw material to form bile acids was cholesterol [35].

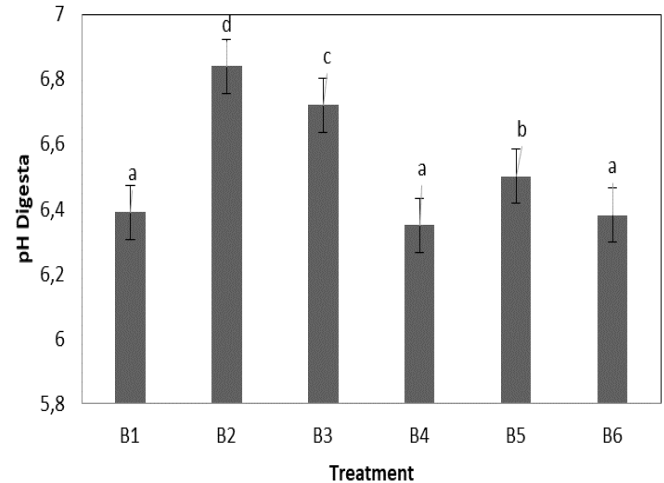


Fig. 5. digesta pH mice in each treatment. Normal fed with standard AIN 93 M (B1), hypercholesterolemia fed with standard AIN 93 M without treatment (B2), hypercholesterolemia fed with natural Cavendish Jepara 30 EBP flour EBP flour (B3), hypercholesterolemia fed with blanched Cavendish Jepara 30 EBP flour (B4), hypercholesterolemia fed with canna starch-based food bar which was substituted with flour Cavendish Jepara 30 EBP (B5), and hypercholesterolemia fed with standard AIN 93 M with medication of simvastatin 3ml.200⁻¹ g (B6). The averaged values followed by different letters was significantly different (p <0.05).

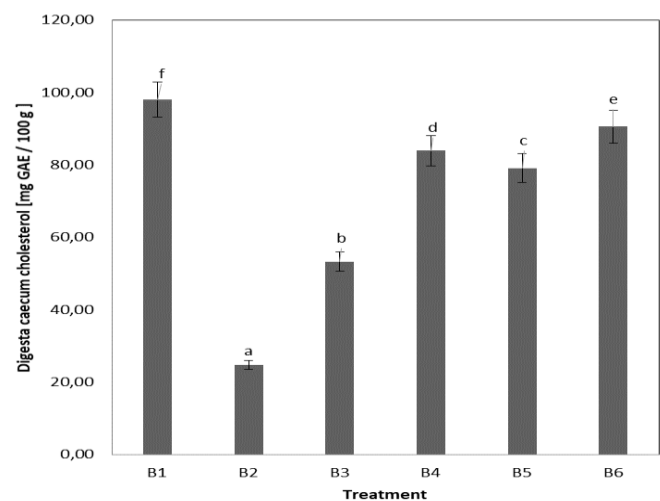


Fig. 6. Cholesterol in caecum digesta of mice in each treatment. Normal fed with standard AIN 93 M (B1), hypercholesterolemia fed with standard AIN 93 M without treatment (B2), hypercholesterolemia fed with natural Cavendish Jepara 30 EBP flour EBP flour (B3), hypercholesterolemia fed with blanched Cavendish Jepara 30

EBP flour (B4), hypercholesterolemia fed with canna starch-based food bar which was substituted with flour Cavendish Jepara 30 EBP (B5), and hypercholesterolemia fed with standard AIN 93 M with medication of simvastatin 3ml.200⁻¹ g (B6). The averaged values followed by different letters was significantly different ($p < 0.05$). The high capability of B1 and B6 to produce cholesterol on caecum digesta was allegedly due to both feed diet for both groups had the ability to bind more bile acids. The high content of bile acids and neutral sterols were key determinants of the cholesterol-lowering effect of dietary fiber [6]. Increased excretion of bile acids and neutral sterols were considered as the main determinant for cholesterol-lowering effects of DF. The ability of B4 and B5 to bind cholesterol in caecum digesta (Fig. 6) and to increase the propionic acid of fermentation product in caecum digesta (Fig. 3) proved our previous report stating that B4 and B5 could improve serum lipid profile of hypercholesterolemic mice because it had the ability to bind bile acids [13].

4 CONCLUSION

Diet of blanched EBP flour (B4) and canna starch-EBP based food bar (B5) could produce SCFA and cholesterol in digesta. The presence of propionic acid and cholesterol in caecum digesta of mice indicated that the diet of blanched EBP flour and canna starch-EBP based food bar could lower serum cholesterol. The application of blanched EBP flour and canna starch food bar - EBP was proven to increase propionic acid and cholesterol excretion of digesta caecum so that it was the potentially used for beneficial health purpose.

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