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Research Article

Effects of Complex Carbohydrate from White Jack Bean (*Canavalia ensiformis* L. DC.) Flour after Autoclaving-Cooling Cycles on Short Chain Fatty Acids, Digesta Cholesterol Content and Bile Acid Binding in Hypercholesterolemic Rats

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Abstract

Background and Objective: Complex carbohydrate is a mixture of dietary fibre and starch present in food. The maximum complex carbohydrate content of white jack bean (*Canavalia ensiformis*) can be achieved by treating white jack bean with three autoclaving-cooling cycles. The objectives of this study were to evaluate the effects of complex carbohydrate from white jack bean following autoclaving-cooling on hypercholesterolemic rats and to assess its bile acid binding ability (*in vitro*). **Methodology:** Thirty Sprague-Dawley rats were divided into the following 6 groups: K1, a healthy control group; K2, a negative control group (hypercholesterol); K3, a positive control group (simvastatin); K4, a group administered a diet containing complex carbohydrate flour (5%), K5: a group administered a diet containing complex carbohydrate flour treated with autoclaving-cooling (5%) and K6, a group administered a diet containing complex carbohydrate flour treated with autoclaving-cooling (10%). The intervention lasted 4 weeks. The parameters observed were body weight, lipid profile, short-chain fatty acid (SCFA) profile, digesta cholesterol and bile acid binding ability (*in vitro*). **Results:** The hypercholesterolemic rats of the K6 group, which were fed a diet containing complex carbohydrate flour treated with autoclaving-cooling, exhibited maintained body weight and an improved lipid profile equivalent to those of the K3 positive control group (simvastatin). The rats of the K6 group could produce SCFAs with an acetic:propionic:butyric molar ratio of 50:39:11. The complex carbohydrate flour treated with autoclaving-cooling was able to bind 17.54% of the cholic acid and 32.43% of the deoxycholic acid. The K6 group was able to bind 100.36 mg/100 g digesta cholesterol. **Conclusion:** The K6 group achieved the best results in terms of maintaining the body weight and improving the lipid profile of hypercholesterolemic rats to levels equivalent to those of the K3 positive control group (simvastatin). The K6 group also exhibited an improved SCFA molar ratio with the ability to bind bile acids (*in vitro*) and digesta cholesterol.

Key words: Complex carbohydrates, white jack bean, autoclaving-cooling, hypocholesterolaemia

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cardiovascular disease is currently the largest cause of death globally. Metabolic syndrome is a combination of cardiovascular risk factors such as obesity, dyslipidaemia, hypertension and hyperglycaemia. Obesity is one factor leading to dyslipidaemia, while one key anti-obesity strategy is the consumption of non-starch polysaccharides or fibre[1].

White jack bean (*Canavalia ensiformis* L. DC.) is a legume originally grown in the arid regions of Arizona and Mexico but has now spread to tropical regions[2]. An average production of 7 tons/ha of white jack bean plants have been cultivated in Bengkulu, Lampung, Java, Bali and West Nusa Tenggara of Indonesia. White jack bean is a potential alternative food source because, in addition to having high protein content, it also has a considerable carbohydrate content[3]. Much research has been dedicated to exploring its protein content, although few studies have considered its complex carbohydrate content. White jack bean contains complex carbohydrates, such as 36.9% starch, including 26.1% undigested starch and 10.8% resistant starch. It also contains 17.58% dietary fibre, corresponding to 16.76% insoluble fibre and 0.82% soluble fibre[4].

Autoclaving-cooling cycles can be applied to the white jack bean complex carbohydrate to increase the soluble fibre[5,6] and resistant starch[7,8] content. The autoclaving process causes the breakdown of glycosidic bonds in the dietary fibre so that the components of soluble fibre detach from the insoluble fibre[5]. Benitez *et al.*[5] examined the effect of autoclaving at 115°C for 17-31 min on the skin of onion bulbs. The results showed that there was an increase in soluble fibre and a decrease in insoluble fibre. The ratio of soluble fibre and insoluble fibre changed from 1:3 to 1:2.

Autoclaving-cooling treatment of starch can be used to enhance resistant starch content by gelatinization and retrogradation activity. Gelatinization activity causes the starch granule to expand and become damaged and amylose and amylopectin become diluted into the water. Retrogradation activity causes the starch granules to rebind and under certain conditions, form a crystalline structure of resistant starch, which is difficult to digest. A repeated autoclaving-cooling process can enhance the resistant starch content[9]. Dundar and Gocmen[7] examined the effect of autoclaving-cooling at a temperature of 4°C for 24, 48 and 72 h on high-amylose corn starch (HACS)-resistant starch. Autoclaving-cooling for 72 h produced the highest resistant starch content (30.14%). Ashwar *et al.*[8] studied dual autoclaving-cooling of rice starch and showed that treated rice starches had much higher resistant starch content (30.31-38.65%) than native starches (4.42-10.94%).

Soluble fibre and resistant starch could potentially have hypocholesterolaemic properties through several mechanisms. Dietary fibre consisting of soluble fibre and insoluble fibre can lower cholesterol levels through several mechanisms, such as water binding ability, increased viscosity, bile acid binding ability and cholesterol binding ability and can be fermented in the colon[10]. Resistant starch has hypocholesterolaemic abilities because it will be fermented in the large intestine resulting in short-chain fatty acids (SCFAs)[9]. In fact, one product of the fermentation of dietary fibre in the colon is propionic acid, which is able to inhibit cholesterol synthesis[11].

Several studies have been conducted to investigate the hypocholesterolaemic effects of dietary fibre and resistant starch. There are several studies on the mechanisms of dietary hypocholesterolaemia, including the following: Jimenez *et al.*[12], who examined the effects of dietary fibre hypocholesterolaemia, concluding that the hypocholesterolaemic mechanisms are associated with gel formation, faecal effusion, binding capacity and fermentability. Trinidad *et al.*[11] studied the hypocholesterolaemic effects of coconut fibre. The results showed that the hypocholesterolaemic mechanism was associated with a fermented soluble fibre fraction resulting in SCFAs in the colon. Changes in the propionate/acetate ratio also affect lipid metabolism.

The hypocholesterolaemic effect of resistant starch has been demonstrated in several previous studies. Research by Martinez-Flores *et al.*[13] on the hypocholesterolaemic effects of a diet containing 9.7% resistant starch showed a decrease in total serum cholesterol and very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and low-density lipoprotein (LDL) cholesterol levels due to increased SCFA concentrations in digesta and increased neutral sterol excretion in the faeces. A study by Cheng and Lai[14] showed that rice resistant starch exerted a hypocholesterolaemic mechanism in hypercholesterolemic rats by increasing the levels of the SCFA propionate, which decreased serum and hepatic cholesterol levels. Nugent[15] concluded that propionate is an inhibitor of the 3-hydroxy-3-methylglutaryl-CoA reductase or HMG-CoA reductase enzyme and, thus, effectively inhibits fatty acid synthesis and cholesterol.

The hypocholesterolaemic mechanism is associated with the properties of SCFAs, which are by-products of the fermentation of dietary fibre in the colon[13]. The acetate: propionate ratio of the complex carbohydrate treated with autoclaving-cooling flour fermentation also has an effect on its hypocholesterolaemic ability. According to Damat and Haryadi[16], an increase in the molar ratio of propionate and

butyrate would have implications causing a decrease in the molar ratio of acetic acid and thus, the amount of cholesterol that can be synthesized also decreases since acetic acid is the precursor of cholesterol synthesis in the body. According to Rosida and Rosida[17], acetate and propionate have different effects on fat metabolism. Acetate may act as a precursor of cholesterol synthesis. Acetate can lower glucose and increase cholesterol concentration, whereas propionate may help lower cholesterol concentration.

Martinez-Flores *et al.*[13] reported that a diet containing 9.7% resistant starch was able to lower total cholesterol, VLDL, IDL and LDL due to the increased concentration of SCFAs in the digesta and increased cholesterol excretion. Cheng and Lai[14] reported that administration of resistant starch from rice in hypercholesterolemic rats was able to lower serum and hepatic cholesterol levels by increasing the propionic acid level. Chen *et al.*[18] mentioned that propionic acid could decrease the expression of HMG-CoA reductase as an effect of propionic acid and thus, the rate of cholesterol synthesis in the liver was inhibited. Therefore, the objective of this research was to determine the effect of the white jack bean complex carbohydrate flour after autoclaving-cooling cycles on the body weight and lipid profile of hypercholesterolemic rats and on SCFA content, digesta cholesterol content and bile acid binding ability *in vitro*.

MATERIALS AND METHODS

Dry-harvested white jack bean was collected from Selopura village, Wonogiri district, Central Java, Indonesia. NaOH, HCl, bile acid (cholic acid and deoxycholic acid) were purchased from Food and Nutrition Laboratory, Gadjah Mada University. Dietary cholesterol, simvastatin and cholestyramine were purchased from the Gadjah Mada University pharmacies. Eight-week-old male Sprague-Dawley rats, weighing 180-200 g, were purchased from UPTP (Research Service Unit), Gadjah Mada University, Yogyakarta.

Preparation of the white jack bean complex carbohydrate flour with autoclaving-cooling: White jack beans were soaked in water (1:3 w/v) for 12 h, dehulled, soaked again for 3×12 h and then crushed and dried in a cabinet dryer at 55°C for 16 h, milled and sifted with a 60-mesh sieve. Subsequently, the flour was defatted with hexane (1:3 w/v) for 30 min using a magnetic stirrer and centrifuged for 15 min. The supernatant was separated and the defatted treatment was repeated by centrifuging 3 additional times. The sample was then left for 16 h at room temperature to evaporate the remaining hexane. Next, the flour was deproteinated with 0.1 N NaOH solution at pH 12 by using a magnetic stirrer and centrifuged for 15 min. Deproteination was repeated and the sample was centrifuged twice followed by neutralization with HCl solution 1:3 (w/v) until the pH returned to 7. It was then centrifuged again and the supernatant was separated. The precipitate was dried at 55°C for 16 h, milled and sieved with a 60-mesh sieve.

The complex carbohydrate flour was diluted with water (1:4 w/v) and heated at 90°C for 5 min. The flour solution was then with 3 cycles of autoclaving at 121°C for 15 min and cooling at 4°C for 24 h. The solution was dried in a cabinet dryer at 55°C, milled and sieved with a 60-mesh sieve.

Bioassay of the hypercholesterolemic rat model: Thirty Sprague-Dawley rats were individually caged at 24°C (room temperature) under a 12 h dark/light cycle. The experiments were conducted in accordance with the guidelines and regulations related to a certificate of ethical clearance issued by the Commission of Ethics of LPPT UGM Yogyakarta no. 420/KEC-LPPT/I/2016.

The rats were adapted for 7 days with standard AIN'93 M feed provided *ad libitum*[19]. On the 8th day, the rats were grouped into the following six groups of 5 mice each: K1, a healthy control group; K2, a negative control group (hypercholesterol); K3, a positive control hypercholesterolemic group (simvastatin); K4, a hypercholesterolemic group administered a diet containing complex carbohydrate flour (5%); K5, a hypercholesterolemic group administered a diet containing complex carbohydrate flour treated with autoclaving-cooling (5%) and K6, a hypercholesterolemic group administered a diet containing complex carbohydrate flour treated with autoclaving-cooling (10%). The diets were based on isoenergy and isofibre principles. Hypercholesterolemic induction was performed by feeding a standard diet containing 10 g kg^{-1} dietary cholesterol[20] for 12 days *ad libitum*.

The intervention lasted 4 weeks. Simvastatin was administered according to Katzung *et al.*[21] at a dose of 0.18 mg/200 g. Rat blood was taken once per week from an eye vessel for lipid profile analysis. After 4 weeks of treatment, the rats were anaesthetized and dissected and the caecum was removed. Digesta was taken from rat caecum and then analysed. At this stage of the experiments, the bile acid binding ability was also tested *in vitro*. The chemical composition of the white jack bean complex carbohydrate flour and complex carbohydrate flour treated with autoclaving-cooling is shown in Table 1, while the composition of standard diet and treatment diet for each group is showed in Table 2.

Research analysis

Rat body weight: The rats were weighed once a week throughout the 4 weeks of the intervention.

Lipid profile: Lipid profile was measured using an enzymatic method[22]. The blood was allowed to clot and then centrifuged at 1,500×g for 30 min at 4°C to obtain the serum. Serum samples were analysed by enzymatic colorimetric procedures for total cholesterol (Cat. 60-2/100) and triglycerides (Cat. 59-4/50) using 60-2/10 and 59-4/50 kits, respectively, from Labtest Diagnostica. High-density lipoprotein (HDL) cholesterol was measured after phosphotungstic acid precipitation of VLDL and LDL using kit 13 from Labtest Diagnostica. LDL cholesterol was determined by the Eq. as follows:

$$\text{LDL cholesterol (mg dL}^{-1}) = \text{Total cholesterol - HDL cholesterol - } \frac{\text{Triglycerides}}{5}$$

Short-chain fatty acids: SCFAs were measured by the gas chromatographic method according to Heningsson *et al.*[23] procedure. Rats were dissected and then the digesta from the caecum was extracted. Digesta was centrifuged at 14000 rpm for 15 min. The supernatants were analysed by gas chromatography (GC). The supernatant was injected into a GC column under the following conditions: GP 1200 column 1 percent HPP30, 2 metres column length, 130°C column temperature, 230°C injector detector temperature, nitrogen carrier gas with 1.25 kg cm^{-2} pressure, Shimadzu GC machine GC 8 series.

Bile acid binding ability: The binding ability of bile acid was analysed because bile acid is related to the provision of the complex carbohydrate treated with autoclaving-cooling flour. The hypocholesterolaemic mechanism, i.e., it can increase the excretion of bile acid. The complex carbohydrate treated with autoclaving-cooling flour can lower cholesterol availability because of its ability to bind bile. The binding ability of bile acids (cholic acid, deoxycholic acid) was measured by *in vitro* analysis using the Soral-Smietana *et al.*[24] procedure. For this analysis, 100 mg of flour sample was mixed with 10 mL of each bile acid solution. The solution was prepared in 0.1 mol phosphate buffer at pH 7.6 for each bile acid at a concentration of 2 µmol mL^{-1}. Samples and parallel blank samples were incubated at 37°C for 30 min. Centrifugation was performed at 2000 g for 5 min. Moreover, 50 µL samples were combined with 5 mL of 70% sulfuric acid and 1 mL of fresh furfural solution (2.3 g L^{-1}). All samples were mixed carefully. The absorbance was measured at 510 nm after 80 min. The results were expressed as percent absorption of bile acids.

Table 1: Chemical composition of the white jack bean complex carbohydrate flour and complex carbohydrate flour treated with autoclaving-cooling

Chemical composition	Complex carbohydrate flour	Complex carbohydrate flour treated with autoclaving-cooling
Water (% w.b)	1[illegible]3±0.43[a]	10.68±0.43[a]
Ash (% d.b)	[illegible]0±0.15[a]	0.92±0.04[b]
Fat (% d.b)	[illegible]2±0.00[a]	0.02±0.00[a]
Protein (% d.b)	[illegible]27±0.01[a]	1.85±0.07[b]
Carbohydrate by difference (% d.b)	[illegible]51±0.14[a]	97.20±0.08[b]
Starch (% d.b)	[illegible]67±0.12[a]	68.42±0.39[a]
Resistant starch (% d.b)	14.15±0.07[a]	16.88±0.15[b]
Soluble fibre (% d.b)	14.30±0.07[a]	16.25±0.17[b]
Insoluble fibre (% d.b)	14.54±0.22[a]	12.33±0.12[b]
Total dietary fibre (% d.b)	28.84±0.18[a]	28.58±0.17[a]
HCN (ppm)	3.35±0.53[a]	14.90±1.05[b]

Table 2: Compositions of the standard diet and the treatment diet (g kg^{-1}) for each group

Component (g kg^{-1})	K1	K2	K3	K4	K5	K6
Maizena	620.70	620.70	620.70	540.83	545.44	445.20
Casein	140.00	140.00	140.00	136.89	137.61	135.21
Sucrose	100.00	100.00	100.00	100.00	100.00	100.00
Soybean oil	40.00	40.00	40.00	39.98	39.98	39.96
Carboxy methyl cellulose (CMC)	50.00	50.00	50.00	-	-	-
Complex carbohydrate flour	-	-	-	116.31	-	-
Complex carbohydrate flour treated with autoclaving-cooling	-	-	-	-	109.99	219.97
Mineral mix	35.00	35.00	35.00	33.60	33.99	32.98
Vitamin mix	10.00	10.00	10.00	10.00	10.00	10.00
L-sistin	1.80	1.80	1.80	1.80	1.80	1.80
Kolin	2.50	2.50	2.50	2.50	2.50	2.50
Total	1000.00	1000.00	1000.00	981.91	981.31	987.62
Cal[illegible] (KKal g^{-1})	3.82	3.82	3.82	3.89	3.89	3.87

K1: Group of normal rats, standard diet (healthy control), K2: Group of hypercholesterolemic rats, standard diet (negative control), K3: Group of hypercholesterolemic rats, standard diet and simvastatin (positive control), K4: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour (5%), K5: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (5%), K6: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (10%)

Digesta cholesterol analysis: Digesta cholesterol analysis was done by the Lieberman-Burchard method[25]. About 1 g of digesta plus 10 mL of acetone-alcohol solution (1:1) was heated in boiling water and then cooled at room temperature. The solution was filtered and the filtrate was centrifuged for 15 min at 2,500 rpm. The supernatant was evaporated in a water bath at 100°C to be dried, followed by cooling and dissolving in 3 mL of chloroform solvent plus 3 mL of acetic anhydride solution-concentrated sulfuric acid (30:1). The sample was then homogenized and placed in the dark for 5 min until its colour turned bluish green. A blank solution was composed in the same way. The absorbance was measured at 680 nm. The digesta cholesterol was calculated by comparing the absorbance of the sample with that of the blank solution.

Research design: The bioassay study utilized a completely randomized design with 6 treatments and 5 replications. The treatment at this stage was as follows:

K1: Normal rats, standard diet (healthy control)
K2: Hypercholesterolemic rats, standard diet (negative control)
K3: Hypercholesterolemic rats, standard diet+simvastatin (positive control)
K4: Hypercholesterolemic rats, diet containing complex carbohydrate (5%)
K5: Hypercholesterolemic rats, diet containing complex carbohydrate treated with autoclaving-cooling (5%)
K6: Hypercholesterolemia rats, diet containing complex carbohydrate treated with autoclaving-cooling (10%)

The parameters observed were lipid profile, SCFAs and digesta cholesterol, which were assessed once per week for 4 weeks. The data obtained were analysed by one-way ANOVA with a 0.05% significance level and followed by the Duncan multiple range test (DMRT) using SPSS 23 statistical software.

RESULTS AND DISCUSSION

Rat body weight: The time course of the rats' body weight over 40 days of the intervention is shown in Fig. 1. The results showed that the interventions applied to both the K4 and K5 groups were able to inhibit the increase in the rats' body weight and these interventions were not significantly different ($\alpha = 0.05$). The body weight of the K6 group was maintained such that it was equal to that of the positive control group K3 (simvastatin). These results were similar with those of Martinez-Flores *et al.*[13], who found that hamsters fed a CS-RS diet (a diet containing extruded Cassava starch with resistant starch, 10% coconut oil and 2% cholesterol added) and a CS-OF diet (a diet containing extruded Cassava starch with oat bre, 10% coconut oil and 2% cholesterol added) did not exhibit an increase in body weight.

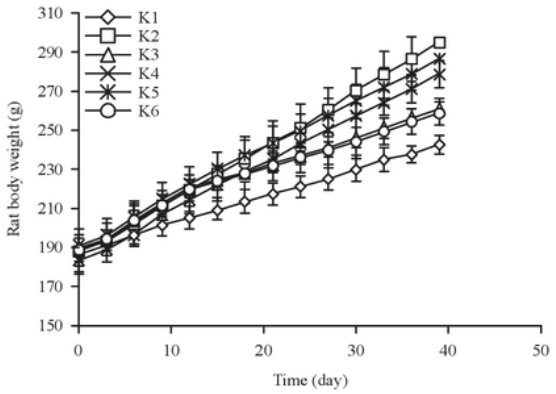

Fig. 1: Time course of the rats' body weight over the 40 days of the intervention
K1: Group of normal rats, standard diet (healthy control), K2: Group of hypercholesterolemic rats, standard diet (negative control), K3: Group of hypercholesterolemic rats, standard diet and simvastatin (positive control), K4: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour (5%), K5: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (5%), K6: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (10%)

The induction of hypercholesterolemia by giving dietary cholesterol rapidly increased the rat's body weight. The provision of dietary fibre and resistant starch was able to prevent a gain in body weight due to the ability to bind sugar, bile acids and cholesterol, thus inhibiting the absorption of nutrients and body weight gain[26].

Serum triglyceride levels: The rat triglyceride levels during the 4 weeks of intervention are shown in Table 3. The results showed that at week 4, the decrease in triglyceride levels in group K5 was significantly greater than that in group K4 ($\alpha = 0.05$). Group K6 at week 4 exhibited a better triglyceride-reducing ability than the positive control group K3 (simvastatin).

The results of decreased serum triglyceride levels were similar to those reported by Macarulla[24]. The results showed that the rats fed a diet of faba bean seeds showed a significantly lower triacylglycerol level (0.73 mg dL^{-1}) than the rats in the control hypercholesterolemic group (1.20 mg dL^{-1}). This is because the dietary fibre of faba beans can reduce the absorption of dietary fat and these rats excreted a significantly higher amount of fat.

Table 3: Triglyceride levels (mg dL^{-1}) of rats during 4 weeks of intervention

	Week				
Group	0	1	2	3	4
K1	75.48^{b}	76.10^{d}	77.66^{d}	78.79^{e}	79.71^{d}
K2	128.62^{a}	128.69^{a}	129.34^{a}	130.00^{a}	130.99^{a}
K3	128.20^{a}	109.21^{c}	99.71^{c}	90.76^{d}	91.87^{c}
K4	126.78^{a}	118.65^{b}	109.20^{b}	105.00^{b}	100.07^{b}
K5	126.08^{a}	122.40^{b}	107.88^{b}	103.49^{bc}	94.36^{c}
K6	127.92^{a}	11[illegible]^{b}	104.79^{b}	100.15^{c}	79.56^{d}

Means followed by the same letter are significan[illegible]fferent at the 5% real level according to the Duncan multiple range test. K1: Group of normal [illegible]s, standard diet (healthy control), K2: Group of hypercholesterolemic rats, standard diet (negative control), K3: Group of hypercholesterolemic rats, standard diet and simvastatin (positive control), K4: Group of hypercholesterolemic rats, diet containing complex ca[illegible]ohydrate flour (5%), K5: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (5%), K6: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (10%)

Table 4: Total cholesterol serum levels (mg dL^{-1}) of rats during the 4 weeks of intervention

	Week				
Group	0	1	2	3	4
K1	87.42^{b}	88.11^{e}	88.93^{e}	89.61^{e}	90.73^{e}
K2	184.11^{a}	184.46^{a}	186.12^{a}	186.71^{a}	188.81^{a}
K3	181.59^{a}	166.76^{d}	143.51^{d}	122.26^{d}	100.38^{d}
K4	181.85^{a}	173.11^{c}	153.13^{c}	147.14^{b}	138.54^{b}
K5	181.85^{a}	170.41^{cd}	158.08^{b}	145.16^{b}	123.98^{c}
K6	182.12^{a}	17[illegible]^{b}	149.42^{c}	132.16^{c}	104.06^{d}

Means followed by the same letter are significan[illegible]fferent at the 5% real level according to the Duncan multiple range test. K1: Group of normal [illegible]s, standard diet (healthy control), K2: Group of hypercholesterolemic rats, standard diet (negative control), K3: Group of hypercholesterolemic rats, standard diet and simvastatin (positive control), K4: Group of hypercholesterolemic rats, diet containing complex ca[illegible]ohydrate flour (5%), K5: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (5%), K6: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (10%)

Table 5: HDL cholesterol levels (mg dL^{-1}) during the 4 weeks of intervention

	Week				
Group	0	1	2	3	4
K1	61.77^{a}	60.49^{a}	60.55^{a}	58.89^{a}	57.67^{a}
K2	25.44^{b}	25.37^{d}	25.30^{e}	24.80^{d}	24.34^{d}
K3	25.44^{b}	38.75^{b}	44.43^{b}	49.59^{b}	52.40^{b}
K4	25.17^{b}	34.15^{c}	37.00^{d}	38.67^{c}	42.79^{c}
K5	24.08^{b}	33.73^{c}	39.21^{cd}	37.93^{c}	50.70^{b}
K6	25.44^{b}	33.8[illegible]	42.69^{bc}	48.12^{b}	50.70^{b}

Means followed by the same letter are significantly d[illegible]ent at the 5% real level according to the Duncan multiple range test. K1: Group of nor[illegible]l rats, standard diet (healthy control), K2: Group of hypercholesterolemic rats, standard diet (negative control), K3: Group of hypercholesterolemic rats, standard diet and simvastatin (positive control), K4: Group of hypercholesterolemic rats, diet containing complex ca[illegible]ohydrate flour (5%), K5: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (5%), K6: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (10%)

The mechanism by which triglyceride levels are reduced is the ability of soluble fibre to trap bile acids and thus increase their excretion. When bile acid excretion is increased, the absorption of fat or triglycerides is disrupted, thus reducing serum triglyceride levels. Dietary fibre and resistant starch can trap digested organic compounds, thereby reducing their bioavailability[26].

Total cholesterol serum: The total cholesterol serum levels of rats during the 4-week intervention are shown in Table 4. The total cholesterol serum of group K6 was equivalent to that of the negative control group K3 (simvastatin). The K5 group exhibited a significantly better ability ($\alpha = 0.05$) to decrease cholesterol serum level[illegible] than the K4 group at week 4. The result showed that the white jack bean complex carbohydrate flour treated with autoclaving-cooling could decreased cholesterol serum levels, which was due to the hypocholesterolaemic effect conferred by the dietary fibre and resistant starch content.

The same result was found by Macarulla *et al.*[27], who stated that hypercholesterolemic rats fed with faba beans can lower serum cholesterol (2.20 mg dL^{-1}) compared with control hypercholesterolemic rats (3.48 mg dL^{-1}). This mechanism is due to the fact that the dietary fibre content of faba beans can directly bind cholesterol, causing cholesterol levels to decrease because of cholesterol expenditure through the faeces.

High-density lipoprotein cholesterol: HDL cholesterol levels during the 4 week intervention are shown in Table 5. Group K5 at week 4 exhibited higher HDL cholesterol levels than group K4. Meanwhile, group K6 did not exhibit significantly increased HDL levels ($\alpha = 0.05$) compared with group K5. These results are more promising than those reported by Macarulla *et al.*[27], who studied the hypocholesterolaemic effects of faba bean in rats, showed that the hypercholesterolaemic group fed with faba beans could maintain the HDL levels because of its dietary fibre content.

Low-density lipoprotein cholesterol: The LDL cholesterol levels in rats over the 4 weeks of the intervention are shown in Table 6. The K6 group exhibited the best ability to lower LDL cholesterol and the level of LDL cholesterol in this group was lowered to a level equivalent to that of the positive control group K3 (simvastatin). However, the decrease in LDL cholesterol in group K5 was not significantly different ($\alpha = 0.05$) from that of group K4.

Table 6: LDL cholesterol levels (mg dL^{-1}) of rats for the 4 weeks of intervention

	Week				
Group	0	1	2	3	4
K1	37.37^{b}	37.69^{d}	38.51^{e}	39.51^{d}	40.60^{d}
K2	75.99^{a}	76.68^{a}	78.96^{a}	80.33^{a}	81.35^{a}
K3	76.40^{a}	65.99^{c}	57.84^{d}	51.80^{c}	50.68^{c}
K4	76.82^{a}	72.49^{ab}	64.98^{b}	60.82^{b}	57.59^{b}
K5	74.19^{a}	71.19^{b}	63.35^{bc}	58.52^{b}	55.19^{b}
K6	73.63^{a}	$6[illegible]^{bc}$	60.07^{cd}	52.79^{c}	49.62^{c}

Means followed by the same letter are significantly different at the 5% real level according to the Duncan multiple range test. K1: Group of normal rats, standard diet (healthy control), K2: Group of hypercholesterolemic rats, standard diet (negative control), K3: Group of hypercholesterolemic rats, standard diet and simvastatin (positive control), K4: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour (5%), K5: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (5%), K6: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (10%)

Table 7: Molar ratio of short-chain fatty acids among rat groups

Group	Acetic acid (% M)	Propionic acid (% M)	Butyric acid (% M)
K1	53 ± 1.91^{b}	37 ± 1.47^{c}	10 ± 0.53^{b}
K2	56 ± 0.58^{e}	35 ± 0.65^{a}	9 ± 0.16^{a}
K3	55 ± 0.69^{d}	36 ± 0.31^{b}	9 ± 0.48^{a}
K4	55 ± 1.28^{d}	36 ± 0.79^{b}	9 ± 0.52^{a}
K5	54 ± 1.82^{c}	36 ± 1.05^{bc}	10 ± 0.80^{ab}
K6	50 ± 3.47^{a}	39 ± 2.89^{d}	11 ± 0.87^{c}

Means followed by the same letter were not significantly different according to DMRT at the 5% significance level, K1: Group of normal rats, standard diet (healthy control), K2: Group of hypercholesterolemic rats, standard diet (negative control), K3: Group of hypercholesterolemic rats, standard diet and simvastatin (positive control), K4: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour (5%), K5: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (5%), K6: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (10%)

The LDL cholesterol result of this study is similar to the results reported by Macarulla *et al.*[27], who showed that the hypocholesterolaemic effects of faba beans fed to rats were achieved by their LDL-reducing ability. The rat group that was fed faba beans had a lower LDL cholesterol level (1.11 mmol dL^{-1}) than the control hypercholesterolemic rat group (2.54 mmol L^{-1}) because faba beans contain dietary fibre that helps reduce LDL cholesterol. Dietary fibre binds cholesterol and bile in the small intestine so that its reabsorption is inhibited. Reduced reabsorbed bile causes the liver to increase LDL catabolism for bile acid synthesis and therefore, blood LDL levels decrease.

Short-chain fatty acid content: SCFA is the result of dietary fibre and resistant starch fermentation in the colon and includes acetic acid, propionic acid and butyric acid. Group K6 produced SCFA with an acetic acid:propionic acid:butyric acid molar ratio of 50:39:11. The propionic acid molar ratio was better than that reported by Nastiti *et al.*[28], who showed that a diet containing 50% jack bean resistant starch flour resulted in SCFA with an acetic acid : propionic acid : butyric acid molar ratio of 66:22:12. The SCFA molar ratio among rat groups is shown in Table 7. The white jack bean complex carbohydrate flour treated with autoclaving-cooling can produce a sufficient amount of propionic acid. It can be concluded that the decrease in cholesterol occurred through the ability of propionic acid to inhibit cholesterol synthesis. Wong *et al.*[29] reported that cholesterol reduction through the prevention of cholesterol synthesis occurs by inhibition of HMG-CoA reductase enzyme activity. This enzyme plays a role in the formation of mevalonate, which is a major product in the formation of cholesterol. By inhibiting HMG-CoA reductase enzyme activity, mevalonate is not formed and thus, cholesterol synthesis is inhibited.

The acetate: Propionate ratio also has an effect on hypocholesterolaemic ability. According to Damat *et al.*[16], an increase in the molar ratio of propionate and butyrate would implicate a decrease in the molar ratio of acetic acid; thus, the amount of cholesterol that can be synthesized also decreases, since acetic acid is the precursor of cholesterol synthesis in the body. According to Rosida and Rosida[17], acetate and propionate have different effects on fat metabolism. Acetate may act as a precursor of cholesterol synthesis, whereas propionate may help lower cholesterol concentration.

***In vitro* bile acid binding ability:** The white jack bean complex carbohydrate flour treated with autoclaving-cooling contains dietary fibre and resistant starch. The white jack bean complex carbohydrate flour treated with autoclaving-cooling was better able to bind cholic acid (17.54%) and deoxycholic acid (32.43%) than the white jack bean complex carbohydrate flour (bind cholic acid, 7.02%; deoxycholic acid, 8.11%). However, the binding abilities of both flours were still below the binding ability of cholestyramine, which can bind more cholic acid (73.68%) and deoxycholic acid (75.68%).

The ability of the complex carbohydrate flour treated with autoclaving-cooling to bind bile acids was affected by the type, chemical composition and treatment of the dietary fibre. Autoclaving treatment has been shown to increase the bile acid binding ability of dietary fibre[30,31] because the microstructure of modified flour becomes more porous after autoclaving-cooling treatment, allowing bile acids to better bind to dietary fibre[32]. Cholic acid is a primary bile acid, whereas deoxycholic acid is a secondary bile acid. Generally, deoxycholic acid binds more strongly to dietary fibre than cholic acid[31,33].

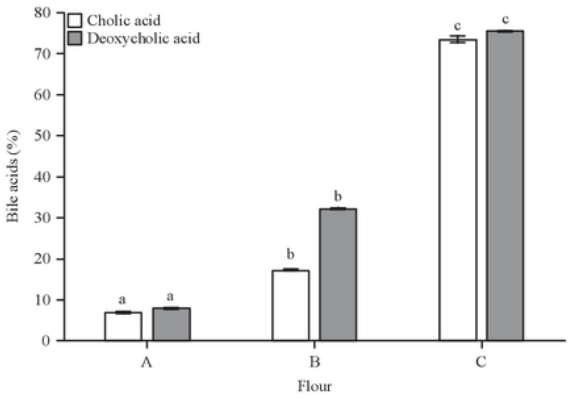

Fig. 2: Ability of complex carbohydrate flour (a), complex carbohydrate flour treated with autoclaving-cooling (b) and cholestyramine (c) in binding cholic acid and deoxycholic acid. Mean followed by the same letter was not significantly different according to DMRT at 5% significance level

Table 8: Cholesterol levels in the digesta after 4 weeks of intervention (mg/100 g)

Group	Digesta cholesterol
K1	13.10±8.13[e]
K2	29.18±3.61[d]
K3	101.83±2.05[a]
K4	69.24±2.32[c]
K5	95.09±3.11[b]
K6	100.36±2.55[ab]

Means followed by the same letter were not significantly different according to DMRT at the 5% significance level, K1: Group of normal rats, standard diet (healthy control), K2: Group of hypercholesterolemic rats, standard diet (negative control), K3: Group of hypercholesterolemic rats, standard diet and simvastatin (positive control), K4: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour (5%), K5: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (5%), K6: Group of hypercholesterolemic rats, diet containing complex carbohydrate flour treated with autoclaving-cooling (10%)

Cholestyramine is a drug that works to lower cholesterol levels in the blood. Cholestyramine works to bind bile acids in the intestine, thus preventing the absorption of bile acids. Cholestyramine and bile acid are then excreted through the faeces, causing a lack of bile acids in the body. To compensate for these deficiencies, the liver increases the conversion of cholesterol to bile acids, causing blood cholesterol levels to decline[34].

The white jack bean complex carbohydrate flour treated with autoclaving-cooling can better bind bile acids because of its higher soluble fibre content. By binding the bile acids through dietary fibre in the intestine and excreting bile acids through the faeces, the liver must continuously absorb cholesterol from the blood to be metabolized and secreted into the gall bladder. As a result, blood cholesterol levels reduce[1]. A comparison of the ability of complex carbohydrate flour, complex carbohydrate flour treated with autoclaving-cooling and cholestyramine to bind bile acid (cholic acid and deoxycholic acid) *in vitro* is shown in Fig. 2.

Digesta cholesterol analysis: The highest digesta cholesterol level was observed in group K6 and this level was equivalent to that in the positive control group K3 (simvastatin). Group K5 was able to better bind cholesterol in digesta than group K4. The autoclaving-cooling treatment caused the microstructure of the complex carbohydrate flour to become more porous[31] such that the complex carbohydrate could bind more cholesterol in the intestine and excrete the cholesterol through the digesta. The cholesterol levels in digesta after 4 weeks of intervention are shown in Table 8.

CONCLUSION

Group K6 exhibited the best hypocholesterolaemic ability and had equivalent metabolic parameters to the positive control group K3 (simvastatin). Thus, complex carbohydrate of white jack bean flour with autoclaving-cooling treatment improved the lipid profile of hypercholesterolemic rats, induced the production of SCFAs, bound bile acid *in vitro* to prevent cholesterol absorption and bound cholesterol in the intestine to increase its excretion through the digesta, thereby reducing the amount of cholesterol absorbed by the body.

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