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FULL PAPER

Red kidney bean (*Phaseolus vulgaris* L.) instant porridge: Effect of isomalto-oligosaccharides and Fibercreme as sucrose replacement on lipid profile improvement in hypercholesterol-induced rats

[1,*]Marsono, Y., [2,3]Putri, R.G., [3]Gunawan, H. and [3]Indrawanto, R.

[1]*Food and Nutrition Laboratory, Faculty of Agricultural Technology, Gadjah Mada University, Bulaksumur, Yogyakarta, Indonesia*
[2]*Post Graduate Program of Food Science and Technology, Faculty of Agricultural Technology, Gadjah Mada University, Bulaksumur, Yogyakarta, Indonesia*
[3]*PT. Lautan Natural Krimerindo (LNK), Mojokerto, East Java, Indonesia*

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Abstract

The aim of the present study was to evaluate the effect of consumption pregelatinized kidney bean porridge with variation of sweetener including sucrose (BKM S), isomalto-oligosaccharides (BKM IMO) and Fibercreme (BKM FC) in hypercholesterolemia rats on the lipid profile, bile acid binding capacity, digesta cholesterol excretion and digesta characters including weight, water content and pH. The diets included standard diet for Negative Control rats (NC diet) and kidney bean porridge diets with different sweeteners: sucrose (BKM S diet), IMO (BKM IMO diet) and fibercreme (BKM FC diet), respectively. Red kidney bean instant porridge substituted 30% of total calories of the diets. All of the diets were formulated based on AIN 93M standard and were fed for 28 days. The result showed that BKM IMO and BKM FC diets improve lipid profile of rats by reducing total cholesterol, LDL cholesterol and triglycerides and increasing HDL cholesterol. In addition, both diets increased cholesterol excretion. *In vitro* measurement of bile acid binding capacity showed that BKM IMO and BKM FC have a higher binding capacity than BKM S. It can be argued that increasing of cholesterol excretion and bile acids binding capacity of these diets are responsible for the improving of the lipid profile. BKM IMO and BKM FC have higher digesta weight and water content but lower pH. It is concluded that substitution of sucrose as sweetener with isomalto-oligosaccharides and Fibercreme in the formulation of pregelatinized kidney bean porridge improve the beneficial health effect of kidney bean porridge.

1. Introduction

Changing in food consumption and modern lifestyle contribute to increase non communicable diseases such as diabetes, coronary heart disease and stroke. WHO estimated that 17.9 million people died in 2019 due to CVDs, representing 32% of all global death (WHO, 2021). Dietary fiber has been recommended as a food component to decrease LDL cholesterol (LDL-C) the major risk factor for atherosclerotic cardiovascular disease. Novel fibre such as Isomalto-oligosaccharide (IMO) and resistant maltodextrin (RMD) is currently developed to anticipate the increasing need of dietary fibre.

Isomalto-oligosaccharide or IMO is a partially digestible oligosaccharides, composed of α (1-4) and α (1-6) glycosidic linkages (Hu *et al*., 2016). The glucosil saccharides with α-(1→6) bond such as isomaltose, pannose, isomaltotriose, which is resistant to digestive enzymes (Kohmoto *et al*., 1992). The indigestible component of IMO was pass through the small intestine and was fermented by colonic microflora produce SCFA in the colon (Wu *et al*., 2017). Health Canada (2012) suggested that IMO is a probiotic having calorie of 2.4 kcal/g and can be used as low-calorie sweetener.

Health effect of IMO has been investigated. Yen *et al*. (2011) reported that IMO supplementation into a low-fiber diet improved colonic microflora profile as shown by increasing fecal bifidobacteria, lactobacilli and bacteroides but decreasing the clostridia counts. Wu *et al*. (2017) suggested that chain length of IMO affect the growth of good microflora and level of SCFA production which were higher in long chain IMOs. Wang *et al*.

*Corresponding author.
Email: *yustimar@ugm.ac.id*

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(2001) reported that intake of 30 g IMO for four weeks reduced total cholesterol and triglyceride 17.6% and 18.4%, respectively, and increase 39.1% of HDL-Cholesterol (HDL-C).

IMO has been developed into a multipurpose creamer (Fibercreme) as a high fiber-low calories product consist of 61.6% isomalto-oligosaccharide (PT. Lautan Natural Krimerindo, 2017). In study with experimental animals, it was reported that cellulose substitution with Fibercreme in the diet lower total cholesterol (46%), LDL-C (43%), triglycerides (15%) and increase HDL-C (108%) (Marsono *et al*., 2020). Therefore IMO and Fibercreme (FC) can be used as a low-calorie sweetener with IMO having a half-sweetness level of sucrose (Yoo *et al*., 2006) and Fibercreme theoretically having a sweetness level of about 30.8% sucrose. These studies indicate that both IMO and FC might have a potential effect on the lowering cholesterol when the products were used as part of the food formula.

Red kidney bean is variety of common bean (*Phaseolus vulgaris* L.) rich in dietary fiber but have a hard texture. To overcome this weakness, raw beans were pregelatinized in high pressure cooker, dried and milled to get pregelatinized kidney bean flour (Ridha, 2009). It has been investigated that formulation of pregelatinized kidney bean porridge using the variation of sweetener including sucrose, IMO and Fibercreme (Putri *et al*., 2020). The study evaluated the physical and organoleptic properties of the porridge, but its nutritional properties have not been reported. The aim of the present study was to evaluate the effect of consumption pregelatinized kidney bean porridge with variation of sweetener including sucrose (BKM S), isomalto-oligosaccharides (BKM IMO) and Fibercreme (BKM FC) in hypercholesterolemia rats on the lipid profile, bile acid binding capacity, digesta cholesterol excretion and digesta characters including, weight, water content and pH.

2. Materials and methods

2.1 Material

Main material, red kidney bean (*Phaseolus vulgaris* L.) was obtained from traditional market in Yogyakarta. Isomalto-oligosaccharide (IMO) and Fibercreme (FC) was obtained from PT. Lautan Natural Krimerindo (LNK), Mojosari, Indonesia well packed with its compositional data. Additional material for porridge formulation including sucrose, skim milk and vanilla were purchased from local supermarket in Yogyakarta, Indonesia.

2.2 Instant porridge formulation

Formulation of red kidney bean instant porridge and its characteristics has been reported in the previous publication (Putri *et al*., 2020). Red kidney bean porridge is prepared using pregelatinized kidney bean (*Phaseolus vulgaris* L.) flour as the main component. The composition of instant porridge basal formula including pregelatinized red kidney bean flour (76.2%), skim milk (13.6%), sucrose (10%) and flavoring agent (0.2%). Three variations of porridge were provided including BKM S, BKM IMO and BKM FC with different sweetener of sucrose, IMO (isomalto-oligosaccharide) and Fibercreme, respectively. The formulas were adjusted to have the same level of sweetness, based on sweetness equivalent of IMO (50%) and fibercreme (30.8%) to sucrose. The sucrose, IMO and fibercreme in 100 g formula of the red kidney bean instant porridges are 10 g, 20 g and 31.6 g, respectively (Putri *et al*., 2020).

2.3 Bioassay experiment

A total of twenty-five *Wistar* rats, weighing from 180 to 200 g, aged 2-3 months were obtained from the Center for Food and Nutrition Study, Gadjah Mada University. The rats were housed in individual cage with natural light-dark cycle and stable ventilation at room temperature (25-30°C) with free access to food and water. After 5 days of environmental acclimation 5 rats were fed with AIN93M standard diet (Healthy Control = HC) and 20 rats were fed with hyper cholesterol diet for 7 days to induced hyper cholesterol. The formula of hyper cholesterol diet was similar to standard diet with addition of 10 gr cholesterol and 2 g sodium cholate per 1000 g diet (Reeves *et al*, 1993). After hyper cholesterol induction, the rats were then divided into four groups of 5 rats and were fed standard diet (Negative Control = NC group) and kidney bean porridge diets with different sweeteners: BKM S group, BKM IMO group and BKM FC group, respectively. Red kidney bean instant porridge substituted 30% of total calories of the diets. The diet compositions were presented in Table 1. The experimental procedures involving the use of animals were approved by the Ethical Clearance Comission for preclinic research of Gadjah Mada University (00119/04/LPPT/IX/2017).

2.4 Analytical methods

2.4.1 Lipid profile analysis

Total cholesterol analysis: Blood samples were taken from the reorbital vein and then centrifuged (4000 rpm) for 15 mins to separate the serum. Total cholesterol was determined enzymatically using the CHOD-PAP method (Allain *et al*., 1974) weekly during 4 weeks intervention.

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Table 1. Feed composition with red kidney bean instant porridge substitution.

Compound	STD diet (g)	BKM S diet (g)	BKM IMO diet (g)	BKM FC diet (g)
Corn starch	620.7	440.9	479.71	520.38
Sucrose	100	66	0	16
Alpha cell	50	0	0	0
Casein	140	60.41	65.04	84.72
Soy oil	40	35.67	35.14	3.09
Vitamin mix	10	10	10	10
Mineral mix	35	23.45	23.78	23.97
Cholin B	2.5	2.5	2.5	2.5
L-cystine	1.8	1.8	1.8	1.8
BKM S	0	360	0	0
BKM IMO	0	0	403	0
BKM FC	0	0	0	338
Calories/g	3.83	3.71	3.54	3.61
Dietary Fiber (%)	5	6.00	14.26	11.32

STD: Standard (AIN93M), BKM S: kidney bean porridge with sucrose sweetener, BKM IMO= kidney bean porridge with IMO sweetener, BKM FC: kidney bean porridge with Fibercreme sweetener

A total of 10 µL serum was loaded into the test tube, and 1 mL CHOD-PAP cholesterol reagent was added. Serum and reagent were vortexed then incubated at 20-25°C for 20 mins. Absorbance was read at λ = 546 nm. The procedure was performed for blanks and standards. The standard concentration was 200 mg/dL. Total cholesterol level was calculated using the following equation:

$$Blood\ total\ cholesterol\ \left(\frac{mg}{dL}\right) = \frac{Absorbance\ sample}{Absorbance\ STD} \times Cons.STD$$

LDL cholesterol analysis: LDL cholesterol levels pre - and postintervention were measured enzymatically using the CHOD-PAP method (Wieland and Siedal, 1983). A total of 10 µL serum was taken into the test tube, and then, 1 mL CHOD-PAP cholesterol reagent was added. Serum and reagent were vortexed and then incubated at 20-25°C for 20 mins. Absorbance was read at λ = 546 nm. The procedure was performed for blanks and standards. The standard concentration was 100 mg/dL. Total LDL cholesterol level was calculated using the following equation:

$$Blood\ total\ cholesterol\ \left(\frac{mg}{dL}\right) = \frac{Absorbance\ sample}{Absorbance\ STD} \times Cons.STD$$

HDL cholesterol analysis: HDL cholesterol was examined using CHOD-PAP method (Lopes-Virella *et al*., 1977), pre- and postintervention. Blood samples were transferred to an Eppendorf and centrifuged (4000 rpm) for 15 mins until blood serum was obtained. Then, 200 µL of serum was inserted into the test tube, and 500 µL of HDL precipitant was added. The solution was mixed using a vortex and incubated at 20-25°C for 15 mins. After incubation, the supernatant was separated via centrifugation at 3000 rpm for 20 mins. A total of 100 µL supernatant was loaded to the test tube, and 1 mL CHOD-PAP cholesterol reagent was added. The solution was mixed with a vortex and incubated at 20-25°C for 20 mins. Absorbance was read at λ = 546 nm. The procedure was performed for blanks and standards. The standard concentration was 200 mg/dL. Total HDL cholesterol level was calculated using the following equation:

$$Blood\ HDL\ cholesterol\ \left(\frac{mg}{dL}\right) = \frac{Absorbance\ sample}{Absorbance\ STD} \times Cons.STD$$

Triglyceride analysis: Triglyceride levels pre and post intervention were examined using the GPOP-PAP method (McGowan *et al*., 1983). A total of 10 µL serum was transferred to the test tube, and then, 1 mL CHOD-PAP cholesterol triglycerides was added. Serum and reagent were vortexed and incubated at 20-25°C for 20 mins. Absorbance was read at λ = 546 nm. The procedure was performed for blanks and standards. The standard concentration was 200 mg/dL. The triglyceride level was calculated using the following equation:

$$Blood\ triglyceride\ \left(\frac{mg}{dL}\right) = \frac{Absorbance\ sample}{Absorbance\ STD} \times Cons.STD$$

2.4.2 Digesta profile analysis

The weight of the digesta was weighed by analytic scales, digesta water content by AOAC method (AOAC, 1995) and measurement of pH by pH-meter. Cholesterol levels digesta measured with Liebermann-Burchard reaction as done in the previous research (Maryanto and Marsono, 2019). The sample (1 g) was dissolved into 20 mL of 96% ethanol and then the 5 mL filtrate was placed on a water bath until it dry. Filtrate transfered to the test tube and was added chloroform to 5 mL and mixed with 2 mL of vinegar anhydride and 0.1 mL of sulfate. The tube was placed in a dark place for 30 min and measured with spectrophotometer to the blank at λ 620 nm.

Briefly, 0.1 g of dry sample was taken into a 25 mL glass stoppered tube and mixed well with 12 mL ethanol-ether (3:1), shaken vigorously for 1 min and incubated in room temperature for 30 mins. After incubation, the supernatant was separated via centrifugation at 5000 rpm for 3 mins. The filtrate was taken into the beaker glass and evaporated in a water bath until it dried then added 5 mL chloroform was added and 2 mL of vinegar anhydride and 0.1 mL of sulfate was mixed into it. The tube was placed in a dark place for 30 mins and absorbance was measured with spectrophotometer UV-1201 (Shimadzu, Japan) to the blank at λ 620 nm (Abs sample). Similar procedure was conducted for cholesterol standard solution (Conc Std). Equation:

$$Caecal\ digesta\ cholesterol = \frac{Abs\ sample}{Abs\ std} \times Conc\ Std$$

2.4.3 In vitro test of bile acids binding capacity

In vitro test of bile acid binding capacity was

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determined using a method previously reported by Soral-Smietana *et al*. (2000). Standard cholic acid and deoxycholic acid were dissolved in 50 mmol/L phosphate buffer (pH 6.5) to make a 2 mM bile acid solution, which is the same concentration of bile acid in the human body (1.5 – 7 mM). The pH was adjusted to the physiological pH of the duodenum. Then, 100 mg of sample and 10 mL 2 mM bile acid solutions were mixed and the individual substrate solution without sample were used as a blank. Samples and blanks were incubated in water bath shaker (37°C, 120 rpm, 30 mins) and centrifuged for 15 mins. Supernatant of 100 µL was added 5 mL of 70% sulfuric acid and 1 mL of new furfural solution (0.25%). Incubated for 60 mins and was measured with spectrophotometer at λ 510 nm. Duplicate assays were performed for each bile acid assay. Number of bile acids binding capacity (%) was calculated using the following equation:

$$Bile\ acid\ binding\ capacity\ (\%) = \frac{Abs\ blank - Abs\ sample}{Absorbance\ blank} \times 100$$

2.5 Statistical analysis

Mean values of each parameter tested were calculated and the results were expressed as mean ± SD of 5 rats. One way analysis of Variance (ANOVA) was carried out and followed by Duncan's Multiple Range Test (DMRT) at the 95% confidence level, using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1 Total feed intake

Total feed intake for HC, NC, BKM S, BKM IMO, BKM FC during 28 days intervention were 331.4 g; 298.6 g; 306.2 g; 335.0 g; and 355.6 g, respectively. Feed substitution with red kidney bean porridge has no significantly different effect ($p<0.05$) on daily intake. This result indicated that feed substitution of starch with red kidney bean porridge has good palatability.

3.2 Increasing of body weight

Increasing of the body weight among the groups of rats during the experiment can be seen in Figure 1. The figure showed that increasing of the body weight was no significant different among the groups fed with the instant kidney bean porridge diet (BKM S, BKM IMO and BKM FC rats). Negative Control (NC) rats was higher than the rats fed with the BKM IMO and BKM FC diet but was not different with BKM S diet. It might be due to its dietary fiber content of the diet which were higher in the BKM IMO and BKM FC diet (Table 1). It was well known that dietary fiber might inhibit nutrient absorption in the small intestine.

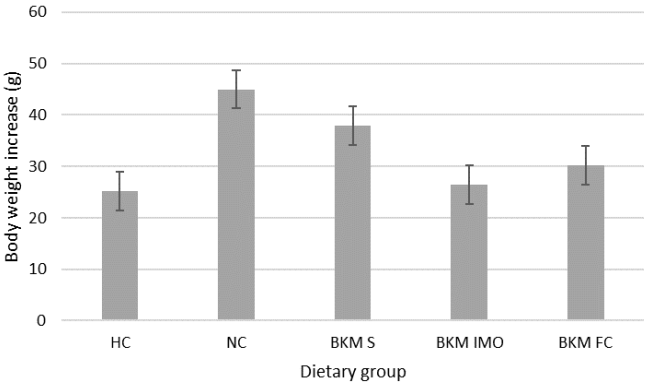

Figure 1. Body weight increase during intervention. HC: healthy control, NC: negative control, BKM S: porridge with sucrose sweetener, BKM IMO: porridge with IMO sweetener, BKM FC: porridge with Fibercreme sweetener.

3.3 Total blood cholesterol

The present study examined the effects of kidney bean porridge diet on lipid profile of hypercholesterolemia rats. Figure 2 shows that after hyper cholesterol-induced treatment, total cholesterol of the NC, BKM S, BKM IMO, and BKM FC rats were significantly increase but not for the HC group. After the rats fed with kidney bean diets total cholesterol decrease gradually until the end of research. The highest reduction of blood cholesterol has found at BKM IMO (67.22%), followed by BKM FC (59.67%) and BKM S (53.81%), while the NC diet decrease total cholesterol 8.18%. Basically, Fibercreme and IMO are high fiber products especially soluble fiber as IMO is an oligosaccharide and Fibercreme consist of 61.6% isomalto-oligosaccharide (PT. Lautan Natural Krimerindo, 2017). Therefore, the higher potential of BKM IMO and BKM FC in lowering cholesterol than BKM S and NC might be due to the higher dietary fiber content (Table 2). Gunnes and Gidly (2010) suggested that there were three major biological mechanism of how soluble fiber reduce cholesterol: prevention in bile salt re-absorption, reduced glycemic response and fermentation product of dietary fiber mainly propionic acid. In this study the possible mechanism lowering total cholesterol BKM IMO and BKM FC was due to prevention of bile salt re-absorption. Table 4 reveals that bile acid binding of BKM IMO and BKM FC were higher than BKM S. Prevention of bile salt re-absorption increases hepatic bile acid synthesis resulted in total cholesterol reduction. In addition, the other possibility of lowering cholesterol was due to the excretion of cholesterol. Table 4 shows that cholesterol excretion was highest in the BKM IMO (3.85 mg/100 g), following with BKM FC and BKM S by 3.43 mg/100 g and 1.44 mg/100 g, respectively. Jesch and Carr (2017) suggested that food components such as phytosterol, soluble fiber, phospholipids and stearic acids inhibit cholesterol absorption. This finding was in line with the previous research by Wang *et al.* (2001), which

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reported that 30 g daily intake of IMO could reduce up to 17,6% of total blood cholesterol. Marsono *et al*. (2020) reported that cellulose substitution with Fibercreme in feed, lower cholesterol levels by 46% in hypercholesterolemia-diabetic rats.

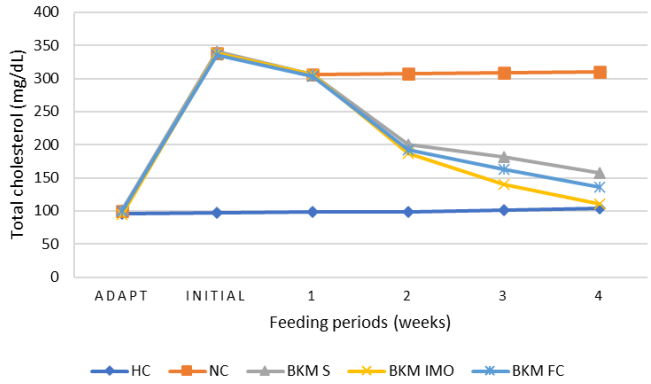

Figure 2. Total blood cholesterol during research. HC: healthy control, NC: negative control, BKM S: porridge with sucrose sweetener, BKM IMO: porridge with IMO sweetener, BKM FC: porridge with Fibercreme sweetener.

3.4 Change in LDL and HDL cholesterol and triglyceride

LDL-C, HDL-C and triglyceride (TG) level were observed before and after treatment. Table 2 shows that lowering of LDL-C level were highest in the BKM IMO (55.02%), followed by BKM FC (44.06%) and BKM S (28.29%). Similar trend in the reduction in TG were found. Reduction of TG in rats fed with BKM IMO, BKM FC and BKM S were 42.08%, 27.36% and 10.91%, respectively. This finding revealed that the higher of dietary fiber content of the diet, the higher of lowering LDL-C and TG level. It was likely that the lower LDL cholesterol in the BKM IMO and BKM FC compared to BKM S was due to the oligosaccharide (IMO) of both diets.

The cholesterol lowering effect of soluble dietary fibre has been known for decades. In this present study, the LDL-C reduction was due to the bile acid binding by dietary fiber. As it is shown in Table 4, bile acid binding of BKM IMO and BKM FC were higher than BKM S. Primary bile acids such as cholic acid and chenodeoxycholic acid are synthesized de novo in the liver from cholesterol. During digestion, bile acids were secreted to the small intestine for the emulsification of lipids. The binding of bile acid increases the loss of bile acids through the feces and consequently reduce the enterohepatic circulation of bile acids to the liver. It will trigger bile acid synthesis in the liver resulting in reduction of cholesterol. Surampudi *et al*. (2016) suggested that dietary fiber increases the bile excretion, leading to reduction of total cholesterol and LDL cholesterol level. Soluble fiber help decrease TG levels by decreasing and slowing down the absorption of fats. Since IMO is soluble fiber it can be argued that the hypocholesterolemic properties BKM IMO and BKM FC are due to its dietary fiber content. The increase of bile acid binding limited the emulsification of lipids with the impact of reducing TG absorption, resulted in lowering plasma TG. Similar findings were reported in the previous research with the oligo fructose (Costa *et al.,* 2015) and with inulin (Causey *et al.,* 2000). It is interesting to note that in contrast with LDL-C and TG, HDL-C increased in the rats fed with BKM IMO, BKM

Table 2. Change in LDL-C, HDL-C, and triglyceride.

Group	Δ LDL-C (%)	Δ HDL-C (%)	Δ Triglyceride (%)
HC	4.83±0.72^{d}	-2.64±0.50^{a}	6.29±1.46^{e}
NC	4.30±1.14^{d}	-6.23±1.31^{a}	2.65±0.59^{d}
BKM S	-28.29±2.30^{c}	66.43±3.69^{b}	-10.91±1.04^{c}
BKM IMO	-55.02±1.94^{a}	173.07±10.21^{d}	-42.08±0.43^{a}
BKM FC	-44.06±1.02^{b}	112.45±5.47^{b}	-27.36±1.15^{b}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significant different (P<0.05). HC: healthy control, NC: negative control, BKM S: porridge with sucrose sweetener, BKM IMO: porridge with IMO sweetener, BKM FC: porridge with Fibercreme sweetener.

Table 3. Digesta profile.

Group	Weight (%db)	Water (%)	pH	Cholesterol (mg/100 g)
HC	5.87±1.20^{b}	83.10±1.02^{e}	6.43±0.02^{a}	4.28±0.07^{e}
NC	2.19±0.16^{a}	34.48±0.62^{a}	6.74±0.02^{c}	1.09±0.08^{a}
BKM S	1.90±0.26^{a}	42.41±1.68^{b}	6.58 ±0.01^{b}	1.44±0.03^{b}
BKM IMO	8.74±1.57^{c}	75.21±1.01^{d}	6.40±0.01^{a}	3.85±0.07^{d}
BKM FC	3.75±0.91^{ab}	65.72±1.30^{c}	6.43±0.02^{a}	3.43±0.04^{c}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significant different (P<0.05). HC: healthy control, NC: negative control, BKM S: porridge with sucrose sweetener, BKM IMO: porridge with IMO sweetener, BKM FC: porridge with Fibercreme sweetener.

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Table 4. Bile acid absorption capacity.

Sample	Cholic acid (%)	Cholic acid (% Cholestyramine)	Deoxycholic acid (%)	Deoxycholic acid (% Cholestyramine)
Standart (AIN 93)	7.24±0.22[a]	9.5	4.35±0.15[a]	6.5
BKM S	16.92±0.83[b]	22.3	16.63±0.45[b]	25.0
BKM IMO	37.52±0.06[d]	49.5	30.94±0.90[d]	46.6
BKM FC	22.56±0.22[c]	29.7	20.55±0.30[c]	30.9
Cholestyramine	75.83±0.43[e]	100	66.41±0.12[e]	100

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significant different (P<0.05). BKM S: porridge with sucrose sweetener, BKM IMO: porridge with IMO sweetener, BKM FC: porridge with Fibercreme sweetener.

FC and BKM S, by 173.07%; 112.45% and 66.43%, respectively. Previous study reported that patients receiving IMO had reduction of total cholesterol and TG by 17.6% and 18.4% but increasing of HDL by 39.1% (Wang *et al.,* 2001). Marsono *et al*. (2020) reported that substitution of cellulose with Fibercreme in the diet fed to hypercholesterol-diabetic rats increase 108% of HDL level but reduce LDL and triglyceride by 43% and 15%, respectively. These current data are also in accord with the previous research conducted by Zhou *et al*. (2015) in which dietary fiber intake over 30 g/day increased plasma HDL-Cholesterol.

3.5 Digesta profile

Digesta profile was observed to evaluate the influence of diet treatment on the colonic content including water content, weight and pH of digesta. In addition, cholesterol content of digesta was measured to evaluate the effects of dietary fiber of the diet on cholesterol excretion. It is found that among the kidney bean diets, BKM IMO has the highest weight and water content, but lowest in pH, followed by BKM FC and BKM S (Table 3). These data indicate that dietary fiber content of the diets plays important role on the digesta profile. Digesta weight represents amount of undigested food and microorganism mass (Marsono, 1995). The weight of digesta also affected by water content. It has been suggested for a long time that one of the important physiological effects is high water holding capacity. The pH of digesta reflects the fermentability of dietary fiber in the colon. The lowest pH of BKM IMO diets represent the highest fermentability due to the highest content of dietary fiber in the diets (Table 1).

Total cholesterol excretion was highest in the BKM IMO (3.85 mg/100 g) followed with BKM FC and BKM S by 3.43 mg/100 g and 1.44 mg/100 g, respectively. It can be argued that dietary fiber content is responsible for the excretion of the cholesterol because as shown in Table 1, BKM IMO diet contain the highest dietary fiber. A review conducted by Jesch and Carr (2017) concluded that soluble fibers have an important role in cholesterol excretion.

3.6 Bile acid binding capacity with in vitro method

Bile acid binding capacity of the diets in this study were determined to prove the hypothesis that it is responsible for lowering cholesterol. Bile acid binding capacity was measured by *in vitro* analysis (Soral-Smietana *et al*., 2000). In brief, the sample (100 mg) was mixed with 10 mL of solution of each bile acid (cholic acid and deoxycholic acid). The solutions were prepared in 0.1 mol phosphate buffer pH 7.6 for each bile acid in 2 µmol/mL concentration. The sample and parallel blank samples were incubated at 37°C for 30 mins. Then the mixtures were Centrifugated at 2000×*g* for 5 mins. The sample (50 µL) was combined with 5 mL of 70% sulphuric acid and 1 mL of freshly-prepared furfural solution (2.3 g/L) with careful mixing of the whole sample. Absorbance was measured at 510nm after 80 mins. The results were expressed as per cent of bile acid absorption capacity. Cholestyramine was used as reference standard. Table 4 shows that cholic acid absorption capacity of BKM S, BKM IMO and BKM FC was 22.3%, 49.5% and 29.7% respectively. Whereas deoxycholic acid absorption capacity of BKM S, BKM IMO and BKM FC are 25.0%, 46.6% and 30.9%, respectively. It was clear that replacement of sucrose with IMO or FC in the red kidney bean porridge increase the capacity of bile acid absorption. Since IMO and FC are high in dietary fiber it can be argued that higher bile acid binding for BKM IMO and BKM FC compared to BKM S was due to its dietary fiber content. As the IMO content in the BKM IMO is higher than in the BKM FC the effect on the bile acid absorption capacity is inline. This finding may explain of how BKM IMO and BKM FC improve the lipid profile of rats as shown in Figure 2 and Table 2.

Research about bile acid binding capacity of IMO or other oligosaccharides are still limited. Previous research on FC suggested that cholic acids and deoxycholic acid binding capacity of rat diet with FC as a source of dietary fiber were 41.25% and 14.75% of cholestyramine, were higher than the standard diet (Marsono, *et al*., 2020). Kahlon and Smith (2007) reported about bile acid binding capacity of fiber from

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various fruits like banana, peach, pineapple, grapes, pear, apricot, and persimmon. The bile acid binding capacity of these fruits were varied from 1.54 mmol/100 mg to 10.4 μmol/ 100 mg dietary fiber.

4. Conclusion

The result showed that BKM IMO and BKM FC diets improve lipid profile of rats by reducing total cholesterol, LDL cholesterol and triglycerides and increasing HDL cholesterol. In addition, both diets increase cholesterol excretion. *In vitro* measurement of bile acid binding capacity showed that BKM IMO and BKM FC have a higher bile acid binding capacity than BKM S. It can be concluded that increasing of cholesterol excretion and bile acids binding capacity of these diets are responsible for the improving of the lipid profile. BKM IMO and BKM FC have higher digesta weight and water content but lower pH. It is concluded that substitution of sucrose as sweetener with isomalto-oligosaccharides and Fibercreme in the formulation of pregelatinized kidney bean porridge improve the beneficial health effect of kidney bean porridge.

Conflict of interest

The authors declare no conflict of interest.

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