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The Effect of Autoclaved – Cooled Jack Bean (*Canavalia ensiformis* (L.) DC.) High RS-4 Starch on Lowering Glucose Level and Characteristics of Digesta of STZ-NA Induced Type-2 Diabetes Mellitus Rats

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Abstract

Jack bean is one of local beans known to contain natural resistant starch (RS). To increase its RS content, four cycle autoclaving – cooling treatment modification was applied to generate resistant starch type 4 (RS-4) which refers to RS obtained from modification. RS is able to improve blood glucose level of diabetic individuals. This research aimed to measure the effect of jack bean autoclaved – cooled starch (AC starch) diet on blood glucose level and digesta characteristics of type-2 diabetic Sprague Dawley rats in vivo. Bioassay analyses showed that jack bean AC starch consumption decreased blood glucose level at range of 96.05 mg/dl – 135.97 mg/dl. The diet was also able to increase short chain fatty acids (SCFA) production in form of acetic acid, propionic acid, and butiric acid, to increase total weight and moisture content of digesta, while reduce digesta pH, as well as to inhibit glucose absorption. Diet of 50% high RS jack bean AC starch showed the best results in all glucose metabolism parameters for type-2 diabetic animals.

Keywords: jack bean starch, resistant starch type-4, type-2 diabetes mellitus, blood glucose, short chain fatty acids (SCFA)

Introduction

Type-2 diabetes mellitus (DM-2) is a metabolic system disease characterized by chronic hyperglychemia caused by inability of insulin target cells to normally response to insulin (insulin resistance). Several researches indicated that food with high resistant starch might be able to prevent or to control type-2 diabetes mellitus (Fuentes-Zaragoza et al., 2010; Sajilata et al., 2006; Nugent, 2005). In the effort to control the increasing rate of DM-2 insidence in Indonesia, one of potential local food source to be utilized is jack bean (*Canavalia ensiformis* (L.) DC.), which is known for its natural resistant starch content. Sridhar and Seena (2006) reported that it contains approximately 24.7 – 36.9% starch which consist of 26.1% digestible and 10.8% resistant starch. Resistant starch (RS) is the undigestible fraction of starch by digestive enzymes (α-amylase) in small intestine, but still digestible as it can be fermented by colon microflora then generating short chain fatty acids (Englyst et al., 1992). Based on formation mechanism, resistant starch can be classified into RS-1, RS-2, RS-3, RS-4, and RS-5.

RS-4 is particularly formed through modification, either chemically or physically,

to increase starch content which is resistant against amylase (Maningat et al., 2013). Resistant starch (RS) of jack bean can be improved through several methods, such as autoclaving continued with cooling. During this process, heated starch dispersion generates soluble amylose and starch gel. After cooling, gel is retrogradized which lead to crystal structure formation. During retrogradation, amylose reunites to form strong crystalline structure (Haralampu, 2000). Mentari (2016) mentioned that 4 cycles autoclaving-cooling on jack bean starch was able to increase resistant starch content by 23.44%.

Resistant starch (RS) is physiologically fermentable by colon microflora to produce short chain fatty acids (SCFA), such as acetic acid, propionic acid, and butyric acid, which play important role in insulin sensitivity improvement, hence lower blood glucose in diabetic patient. Other physiologic effects of RS in human body are improvement of glycemic and insulin response, improvement of colon health, lipid profile, satiety, reduction of energy intake, glucose absorption inhibition, enhancement of micronutrient absorption, in addition to its prebiotic property (Nugent., 2005; Sajilata et al., 2006; Fuentes-Zaragoza et al., 2010).

This research was aimed to measure the effect of autoclaved – cooled jack bean starch (AC starch) diet on blood glucose levels and digesta characteristics of STZ-NA induced DM-2 Sprague Dawley rats.

Materials and Methods

Materials

Jack bean was obtained from farmers in Tulakan, Wonogiri District, Jawa Tengah Province. Thirty 8 weeks-old Sprague–Dawley male rats (Center of Food and Nutrition Study, UGM, Yogyakarta) weighing 180-200 gram were used. After acclimated for 1 week, each animal was housed in transparent single cage in room temperature (27 ± 1°C) with a 12 hours light-12 hours dark cycle, fed with AIN 93 feed (Reeves et al, 1993) contained vitamin-mineral mix and water was provided *ad libitum*. All protocols were approved by the Pre-clinical Ethical Clearance Commission of Integrated Laboratory for Research and Experiment, Universitas Gadjah Mada.

Research instruments consisted of pH meter, 60 mesh shaker, "Philips" blender, electric stove, 4^{o}C cool room, autoclave, cabinet dryer, muffle (Advantec-FUW 220), oven (Memmert), digital scale "OHAUS", vortex (MSI Minishaker), centrifuge (Damon/IEC Division IEC UV Centrifuge), water bath, microlab, rats cages and their equipment, a set of blood specimen collection tools, and glass analysis tools.

Methods

Preparation of Autoclaved – Cooled Jack Bean Starch (AC Starch)

High RS jack bean AC starch was prepared according to the method by Murdiati *et al.* (2014), initially started by preparation of jack bean powder. After 12 hours of soaking and skin removal, jack bean was re-soaked for another 36 hours to remove its HCN content; water was changed every 12 hours. The bean was crushed and dried in **±** 55^{o}C cabinet dryer, ground, and then shaken using 60 mesh sieve.

The next stage was jack bean starch extraction based on the method by Hoover *et al*. (1985) with slight modification. Jack bean powder was mixed with water (water : powder = 3 : 1) until homogenous then filtered; filtrate was collected. Precipitate was re-washed using water for five times, filtered, and filtrate was precipitated for 24 hours. Wet starch precipitate was obtained after supernatant removal, and then subjected to deproteination which conducted based on the method previously applied by Cornfine et al. (2010). Deproteination was performed three times using 0.1 N NaOH, and precipitate

obtained from the third stage was removed from filtration, prior to HCl 0.1 N addition to reach pH 7. Deproteinized wet starch precipitate was then dried in 55^{o}C cabinet dryer for 24 hours before powdering.

Chemical and Physical Characterization of Jack Bean AC Starch

The analyzed physical characteristics of jack bean AC starch were oil holding capacity and water holding capacity (Chau et al., 1997), while analyzed chemical characteristics were RS analysis (Goni et al., 1996), starch content (Anonim, 1996), and proximate analysis (Anonim, 1996) consist of analysis of protein, fat, moisture, and ash.

In vivo Experiments on Sprague Dawley rats

Blood glucose level and digesta characteristics of Sprague Dawley rats were analyzed in vivo. Thirty 8 weeks-old Sprague–Dawley male rats weighing 180 – 200 g were randomly divided into six groups: healthy rats (STD); DM-2 group (DM-STD), DM-2 group fed with 25% natural jack bean starch diet (DM-KPP25), DM-2 group fed with 50% natural jack bean starch diet (DM-KPP50), DM-2 group fed with 25% AC jack bean starch diet (DM-KPPA25), and DM-2 group fed with 50% AC jack bean starch diet (DM-KPPA25). Type-2 DM was induced by streptozotocin-nicotinamide (STZ-NA) at dosage of 60 mg STZ/kg and 120 mg NA/kg (Szkudelski, 2014).

Result and Discussion

Resistant Starch (RS)

RS is an important compound which able to decrease blood glucose level in DM-2 individuals (Marsono, 2016). From the results, it was obtained that 4-cycles autoclaving – cooling was able to produce RS-4, AC starch had higher RS content than natural starch. Jack bean natural starch contained 10.89 %db RS, 4-cycles autoclaving– cooling modification increased RS content to 12.41 %db. These results were in accordance with another research by Zhao and Lin (2009) that 6-cycles autoclaving - cooling of maize starch increased RS content from 4.1% to 11.2%. Increasing RS content is caused by gelatinization and retrogradation of starch granules during autoclaving-cooling, led to re-association of amylose to form new crystalline structure which more resistant against amylase.

Fasting Blood Glucose

Rats' blood glucose level was analyzed to measure the effect of jack bean AC starch diet during intervention. Blood glucose level of all groups during intervention is presented in **Table 1**. It was indicated that during week-0, all groups suffered from elevated blood glucose.This period was the initial phase of DM induction using STZ-NA prior to jack bean AC starch diet treatment. Healthy rats blood glucose was remained in normal range as they were not injected with STZ-NA for normal glucose metabolism and stable blood glucose.

Significant increase of blood glucose level suffered by other groups of DM-STD; DM-KPP25; DM-KPPA25; DM-KPP50; and DM-KPPA50 in the range of 218.11 mg/dl – 220.16 mg/dl after being induced with STZ–NA indicated that the groups were in DM state. STZ-NA interferes the function of β cells of pancreas, reduces insulin sensitivity which eliminates glucose absorption into tissue, hence blood glucose level increases (Szkudelski, 2012).

Observation of blood glucose level during four weeks intervention showed significant difference in groups fed with jack bean AC starch diet (DM-KPPA25 & DM-KPPA50) compare to DM-STD diabetic group. Hence it indicated that jack bean AC starch diet showed positive effect on blood glucose level of DM-2 rats. In addition, jack bean AC starch group (DM-KPPA) also showed higher rate of

Table 1. Blood glucose level of rats during four weeks intervention

Group	Average Blood glucose level (mg/dl), Week-				
	0	1	2	3	4
STD	63.31± 0.53[a]	63.95± 0.43[a]	65.19± 0.65[a]	66.59± 0.84[a]	69.08± 0.92[a]
DM-STD	220.16± 1.18[b]	220.89± 1.08[d]	222.91± 0.58[e]	228.00± 0.91[f]	232.27± 2.30[f]
DM-KPP25	218.74± 1.44[b]	201.21± 1.43[c]	180.57± 2.14[d]	167.30± 2.24[e]	135.97± 1.94[e]
DM-KPPA25	218.11± 0.83[b]	187.26± 0.83[b]	153.26± 1.25[c]	132.23± 1.85[c]	105.38± 0.60[c]
DM-KPP50	219.61± 0.79[b]	188.63± 0.98[b]	155.96± 1.62[c]	139.77± 2.00[d]	112.94± 2.21[d]
DM-KPPA50	220.08± 1.06[b]	189.43± 0.80[b]	142.91± 1.35[b]	124.86± 2.07[b]	96.05± 1.73[b]

*Different Superscript in the same column showed significant difference at α = 5%. STD = healthy rats; DM-STD = rats DM-2; DM-KPP25 = rats DM-2 fed with 25% jack bean natural starch; DM-KPPA25 = rats DM-2 fed with 25% jack bean AC starch; DM-KPP50 = rats DM-2 fed with 50% jack bean natural starch; DM-KPPA50 = rats DM-2 fed with 50% jack bean AC starch.

blood glucose decrease than natural starch diet group (DM-KPP). Lowest glucose level was obtained by DM-KPPA50 group of 96.05 mg/dl, which indicated that RS content was the influencing factor in blood glucose level decrease. AC starch has higher RS content than natural RS, thus groups treated with AC starch had lower glucose level.

Marsono (2016) noted that glucose level reduction mechanism by RS can be influenced by viscuous RS property and concentration of RS fermentation product in form of short chain fatty acids (SCFA) in colon. Viscuous RS is able to inhibit glucose absorption in small intestine. Besides, RS is also able to prolong stomach emptying therefore impacting in longer satiety, in addition to lower glucose bioavaibility, for its blood glucose lowering effect. RS undigested property by digestive enzymes in small intestine will lead to RS passes and go into colon. RS will be used as substrate or prebiotic by colon bacteria, and being fermented into short chain fatty acids (SCFA) which play important role in insulin sensitivity to reduce blood glucose level of diabetic patient.

Digesta Characteristics

a. Digesta Weight

Data presented in **Table 2** showed digesta weight significant difference of DM-KPPA25, DM-KPP50, DM-KPPA50 treated groups and STD to DM-KPP25 and DM-STD groups, which indicated that RS content feed given to DM-KPPA25, DM-KPP50, and DM-KPPA50 groups increased digesta weight. However, 25% natural starch content given to DM-KPP25 was not enough to increase digesta weight. DM-STD group which not feed with RS diet showed low total digesta weight. The results

Table 2. Digesta Weight of Rats

Group	Total Digesta weight (gram)
STD	5.92± 0.22[b]
DM-STD	4.54± 0.24[a]
DM-KPP25	3.97± 0.10[a]
DM-KPPA25	5.77± 0.30[b]
DM-KPP50	6.45± 0.41[b]
DM-KPPA50	6.04± 0.32[b]

were in accordance with a report by Marsono (1999) showed that higher fiber (RS) consumption led to higher digesta weight compared to non-fiber (RS) group. Beside RS content, moisture content of digesta also influenced digesta weight and volume.

b. Digesta Moisture Content

Based on moisture content data presented in **Table 3**, it was indicated that highest moisture content was obtained by DM-KPPA50 group, as AC starch diet increased digesta moisture content. This was due to higher RS content of AC starch with water binding capacity for its viscous property. The lowest moisture content was obtained by DM-2 group of DM-STD fed with standard feed. DM-KPPA25 and DM-KPPA50 groups had higher moisture content than DM-KPP25 and DM-KPP50, due to DM-KPPA fed with higher RS content than DM-KPP. Higher RS content fed in diet was able to increase water binding to RS, hence increased moisture content digesta.

Table 3. Digesta Moisture content

Group	% Moisture content
STD	58.30± 1.11[d]
DM-STD	37.81± 0.45[a]
DM-KPP25	41.42± 2.72[b]
DM-KPPA25	53.69± 0.89[c]
DM-KPP50	52.57± 0.27[b]
DM-KPPA50	54.60± 1.10[d]

c. pH of Digesta

From results presented in **Table 4**, it was indicated that the highest pH was obtained by DM-STD group. AC starch fed to DM-KPPA25 and DM-KPPA50 group was able to reduce digesta pH level close to normal group (STD). Reduction of pH level was presumably caused by high SCFA content produced by RS fermentation in colon. Higher RS concentration would enhance SCFA level, thus decreased digesta pH. Highest level of RS was fed to DM-KPPA50 intervention group with the lowest digesta pH. The results were similar to those reported by Nielsen (2014), mentioned that RS consumption was able to reduce digesta pH compared to group with no RS diet. Topping (2001) also mentioned that RS passes to colon will induce higher rate of SCFA production, as RS is fermentation substrate for colon microflora, which lead to lower digesta pH.

Table 4. Digesta pH

Group	pH
STD	6.28± 0.13[d]
DM-STD	6.75± 0.04[a]
DM-KPP25	6.40± 0.05[b]
DM-KPPA25	6.41± 0.09[bc]
DM-KPP50	6.39± 0.11[b]
DM-KPPA50	6.39± 0.08[c]

d. Short Chain Fatty Acid (SCFA)

Short chain fatty acid (SCFA) content of digesta was analyzed to measure AC starch on SCFA level. Data is presented in **Table 5**. It was shown that the highest total concentration and pool SCFA was obtained by normal STD group and DM-KPPA50, while the lowest was obtained by diabetic DM-STD group. SCFA level in diabetic rats in DM group digesta showed that the SCFA level was even lower than those of normal rats fed with standar feed (STD). Study by Katoh (1991) reported that diabetic animals had lower response to stimulate SCFA production. In the other hand, DM-KPP25, DM-KPPA25, DM-KPP50, and DM-KPPA50 groups showed higher SCFA pool and total concentration than DM-STD. This

Table 5. Pool SCFA and SCFA concentration

Group	Pool SCFA (mmol)	SCFA concentration (mmol/L)			Total
		Acetic acid	Propionic acid	Butyric acid	
STD	0.2443± 0.010[c]	41.10± 2.35[e]	22.12± 0.86[d]	7.84± 0.21[d]	71.05[e]
DM-STD	0.0252± 0.002[a]	7.73± 0.38[a]	4.58± 0.31[a]	1.53± 0.11[a]	14.85[a]
DM-KPP25	0.0497±0.004[a]	18.22± 1.12[b]	8.47± 0.27[b]	3.53± 0.11[b]	30.23[b]
DM-KPPA25	0.0497± 0.005[b]	31.81± 1.34[d]	10.77± 0.49[c]	5.73± 0.30[d]	48.31[d]
DM-KPP50	0.1282± 0.012[b]	23.85± 0.69[c]	8.85± 0.75[b]	4.88± 0.50[c]	37.58[c]
DM-KPPA50	0.2383± 0.015[a]	40.94± 1.31[e]	20.74± 0.56[d]	10.56± 0.28[e]	72.24[e]

*

Different Superscript in the same column showed significant difference at α = 5%. STD = healthy rats; DM-STD = rats DM-2; DM-KPP25 = rats DM-2 fed with 25% jack bean natural starch; DM-KPPA25 = rats DM-2 fed with 25% jack bean AC starch; DM-KPP50 = rats DM-2 fed with 50% jack bean natural starch; DM-KPPA50 = rats DM-2 fed with 50% jack bean AC starch.

indicated that high RS jack bean AC starch was able to enhance SCFA production, with the highest SCFA pool obtained by DM-KPPA50 group of 0.2383 mmol.

Each SCFA has important role in normalization of blood glucose level due to its ability to improve insulin sensitivity, as fatty acid, reduce lipid capacity and HMGcoA reductase enzyme activity, as well as improve insulin sensitivity. Besides, both acids are also able to increase buffer capacity of colon that maintains colon low pH as colon cancer prevention mechanism. Acetic acid and butiric acid are able to increase AMPK (adenosine monophosphate kinase) level, thus increase glucose utilization led to lower blood glucose concentration. Moreover, butyric acid is also able to increase activity of glucose transporter, showed by GLUT 4 level, to reduce blood glucose concentration.

Conclusion

High RS jack bean AC starch diet was able to reduce blood glucose level of experiment rats down from 218.11 mg/dl – 220.16mg/dl to 96.05mg/dl – 105.38 mg/dl, increase digesta weight, moisture content, SCFA total concentration and pool with main composition of acetic acid, propionic acid, and butiric acid, while reduce digesta pH. Highest concentration of 50% AC starch showed the best result in all observed parameters to improve glucose metabolism in type-2 diabetic individuals.Food containing RS are usefull for people with Type-2 diabetes mellitus (DM-2).

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