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Effect of Fermented Red Bean (*Phaseolus vulgaris L.)* on Body Weight, Profil Lipid and Glucose Serum Sprague *Dawley* Rats Feed High Fat High Fructose Diet

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Abstract:
One of the non-communicable diseases that cause death is metabolic syndrome. Metabolic syndrome is a collection of risk factors for the degenerative disease found in people. Red beans are foodstuffs from the class of nuts that have high fiber content and low glycemic index. Red beans also contain flavonoids, polyphenols, resistant starch, and saponins. That compounds have been found to function as anti-obesity, anti-diabetic, and anti-hyperlipidemia. Red beans fermented is a fermentation product from Rhizopus orizae. Fermentation assumed can improve the nutritional value and health benefits of red beans. The purpose of this study was to determine the effect of red beans fermented on body weight, lipid profile, and blood glucose in Sprague Dawley rats with high carbohydrate and high-fat diets. This research is an experimental design with a pre-post test. Twenty-eight rats were divided into four groups. The first group was given water and standard feed for 28 days. While groups 2, 3, and 4 were fed a high-fat and high-carbohydrate diet for 14 days and then given standard feed and drinking water, red beans fermented, and red beans steamed for 28 days. Weight loss was weighed every week, feed residue was weighed every day, and lipid profiles and blood glucose were measured before and after the intervention. The results showed that red beans and red beans steamed to lose weight, blood glucose, and lipid profiles were significant (p <0.05). Steam red beans have anti-obesity, anti-diabetic and antihiperlipidemia effects that are more effective in improving the condition of metabolic syndrome than the fermentation of red beans.

Keywords: *Red beans fermented, lipid profile, blood glucose*

1. Introduction

Disease causes of death in the world, especially in developing countries, have changed from infectious diseases to non-communicable diseases. According to data from the World Health Organization (WHO) in 2008 for 63% of deaths in the world are caused by non-communicable diseases such as heart disease, diabetes, cancer, and chronic respiratory. The death rate is expected to rise by 15% between 2010 and 2020 (WHO, 2011).

One of the non-communicable diseases that cause death is metabolic syndrome. Metabolic syndrome is a collection of risk factors for the degenerative disease found in people. WHO (1999) defines metabolic syndrome as glucose intolerance, impaired glucose tolerance or diabetes mellitus, and insulin resistance to two or more of the following circumstances, hypertension or blood pressure> 160/90 mmHg, plasma triglycerides ≥150 mg/dL, and low levels of HDL is <35 mg/dL in men or <39 mg/dL in women, central obesity as the ratio of waist circumference> 0.90 in men or> 0.85 in women, or microalbuminuria is the rate of excretion μgm ≥20 urinary albumin/minute or the ratio of albumin/creatinine ≥ 30 μgm/mg.

Based on the International Diabetes Federation (2006), the prevalence of metabolic syndrome in the world in the adult population is estimated to be around 20-25%, and mortality to half of that amount. People with metabolic syndrome can stroke and heart attack three times more often than people without metabolic syndrome. While in Indonesia, the prevalence of metabolic syndrome was not yet known. Data Basic Health Research in 2007 states that the prevalence of obesity in the population> 15 years was 10.3% (Ministry of Health, 2007). While Data Basic Health Research in 2013 states that the prevalence of hypertension and diabetes mellitus are 9.4% and 1.5%, respectively (Ministry of Health, 2013). Thus, the high incidence of metabolic syndrome in the world or in Indonesia marks the rising incidence of degenerative diseases.

Red beans are foodstuffs from the class of nuts that have high fiber content and a low glycemic index and has been widely studied for their hypoglycemic effect. 100 grams of dried red beans contained 6.9 mg of fiber (Atchibri et al., 2010). The fibers may form a gel with high viscosity, which can slow down glucose absorption. Red beans have a glycemic index of 26, which is low and is able to reduce blood glucose (Marsono et al., 2002). Marsono et al. (2003) reported that red beans have a viscosity greater than soybeans but lower glucose absorption, which causes lower blood glucose than soybeans.

Red beans contain potent anti-hyperglycemic flavonoids (Atchibri et al., 2010). Flavonoid is an antioxidant, and antioxidants are needed by patients with diabetes mellitus because hyperglycemia increases free radicals (Setiawan, 2005). Antioxidants can prevent, delay or slow the damage caused by free radicals that cause decreased sensitivity pancreas to produce insulin hormone (Gordon et al., 1997).

Red beans also contain resistant starch and saponin. Substances contained in red beans can potentially improve the lipid profile (Ramirez-Jimenez et al., 2015). Saponin compounds also proved useful as anti-diabetic (Elekofehinti, 2015). The processing of red beans such as heating and fermentation can affect the nutrient content therein. Digestibility and bioavailability of the active compound content of fiber, one of which is an unsaturated fatty acid, increased with the ripening process. The content also has the potential to be a hypocholesterolemic agent (Utari et al., 2010). The benefits of red beans and their processed products are great for health, especially for degenerative diseases. Adding or increasing red bean consumption in the daily diet can be an alternative diet.

Red bean processing innovations continue to evolve to get various benefits therein. Previous research tried to process the beans into milk and yoghurt and then looked at its effect on blood lipid profile hypercholesterolemia rats. However, this has not been researched on red bean fermentation. Therefore, this study has an objective to determine the effect of red bean fermentation on body weight, lipid profile, and blood glucose levels in *Sprague Dawley* rats fed a diet high in fat and high in carbohydrates.

2. Materials and Method

2.1. Materials

Red beans used in this study were obtained from a traditional market in Yogyakarta. Fermented red bean processing begins with soaking for 8 hours, then boiling at a temperature of 100°C for 30 minutes twice. After cold, red beans were inoculated with *Rhizopus orizae* and incubated for 36 hours. Steaming red beans begins with soaking for 8 hours and then steamed at a temperature of 70°C for 2 hours.

2.2. Research Design

This research is an experimental research design – pretest-posttest control group design. The study was conducted at the Laboratory of Food and Nutrition Center for Inter-University (PAU), UGM, in April and May 2016. The subjects were 28 Sprague-Dawley rats (150 g-200 g) that were 2 months old. Animals were reared in individual cages measuring 30x20x20 cm. After adaptation for 7 days, the rats were divided into four groups:

- Negative Control (NC) group, which was given a standard diet,
- Positive Control group (PC),
- The red beans fermented (RBF) group, and
- The red beans steamed (RBS) group, which was given a high-fat and high-carbohydrate diet for 14 days.

Further intervention is performed for 28 days. NC and PC group was given a standard diet AIN-93m, the RBF group was given a standard diet and red beans fermented, and the RBS group obtained a standard diet and red beans steamed.

A high carbohydrate and high-fat diet are given in the form of fructose liquid and beef fat. High carbohydrates and high-fat diet mixed with the standard feed composition refer to the AIN-93 (Reeves et al., 1993), which has been modified by adding 180 grams of beef fat and 58.5 grams of sucrose, as seen in table 1.

No	Composition	Amount (g)			
		Standard	High Fat High Carbohydrate	Red Beans Fermented	Red Beans Steamed
1.	Corn starch	620,7	440,66	424,07	418,29
2.	Casein	140	140	71,26	77,55
3.	Sucrose	100	41,5	100	100
4.	Soy oil	40	40	35,37	34,86
5.	Fiber	50	50	50	50
6.	Mineral-Mix	35	35	35	35
7.	Vitamin	10	10	10	10
9.	*Cholin*	2,5	2,5	2,5	2,5

No	Composition	Amount (g)			
		Standard	High Fat High Carbohydrate	Red Beans Fermented	Red Beans Steamed
10.	Beef fat	-	180	-	-
11.	Fructose	-	58,5	-	-
12.	Red beans fermented flour	-	-	270	-
13.	Red beans steamed flour	-	-	-	270
	Total (g)	1000	1000	1000	1000
	Energy (kcal)	3601	4188,6	3650,93	3652,17

Table 1: Composition of Feed Standard AIN-93 M, High-Carbohydrate High-Fat Feed and Feed the Intervention

2.3. Determination of Lipid Profile and Blood Glucose

Rat blood sampling was performed on the re-orbital plexus. Blood analysis was done 2 times that is before intervention and after intervention enzymatically. Blood glucose levels were analysed using the GOD-PAP (glucose oxidase-phenol aminophenazone) method, while the blood lipid profile was analysed using the CHOD-PAP (cholesterol oxidase-phenol aminophenazone) and GPO-PAP (glycerol phosphate oxidase-phenol aminophenazone) method.

2.4. Statistical Analysis

Data were analyzed using SPSS software. Research data were analysed using One Way ANOVA followed by Tukey's test as the posthoc test.

3. Result and Discussion

3.1. Proximate Analysis

Proximate analysis was conducted to analyze the chemical content of the samples used. Table 2 shows that red beans fermented flour had significantly ($p<0,05$) higher protein content and lower fat than red bean steamed flour. However, the ash content in red beans fermented flour is lower than in red bean steamed flour. According to Astawan et al. (2015), fermentation will increase the proportion of protein and reduce fat levels.

Sample	Composition				
	Water (%wb)	Ash (% db)	Fat (% db)	Protein (% db)	Carbohydrate (% db)
Red beans fermented flour	5,10±0,08	2,91±0,40	1,81±0,07	26,83±0,50	68,45±0,39
Red beans steamed flour	6,47±0,16	4,04±0,04	2,04±0,004	24,73±0,21	69,20±0,25
P	0,008	0,001	0,042	0,032	0,151

Table 2: Results of the Proximate Analysis Red Beans Fermented and Red Beans Steamed

3.2. Feed Intake

During the intervention period, there were significant differences in feed intake between the groups each week (Table 3). Feed intake in the NC group was stable, and the PC group increased in the second week. The RBF group tended to decrease on a weekly basis, while the RBS group decreased during the third week and increased in the fourth week. An average sequence of the rat feed intake ranged from the smallest group of NC, RBF, RBS, and PC.

Group	Feed Intake (gram)			
	Week-1	Week-2	Week-3	Week-4
NC	$11,41±0,85^{a}$	$11,26±0,54^{a}$	$11,33±0,65^{a}$	$11,39±0,47^{a}$
PC	$12,89±0,34^{b}$	$14,06±0,24^{c}$	$14,02±0,13^{b}$	$13,86±0,25^{c}$
RBF	$12,16±0,49^{a}$	$11,92±0,55^{ab}$	$11,37±0,58^{a}$	$11,12±0,39^{a}$
RBS	$12,21±0,31^{ab}$	$12,10±0,42^{b}$	$11,04±0,59^{a}$	$12,37±0,31^{b}$
P	0,000	0,000	0,000	0,000

Table 3: Feed Intake of Rats

3.3. Weight Gain

The red beans steamed group had a mean increase lower than the other group (21.86 ± 1.57 grams). While the red beans fermented group had a high difference that is 22.57 ± 2.23 grams, a negative control group of 18.86 ± 1.95 g, and the positive control group had the greatest changes in body weight, which was 35.86 ± 1.25 grams (Table 3).

Time	Group				P
	NC	PC	RBF	RBS	
Week 1- Week 0	3,00 ± 1,16^{a}	4,71 ± 0,95^{a}	4,71 ±1,11^{a}	4,00 ± 1,73 ^{a}	0,059
Week 2- Week 1	-0,43± 1,40^{a}	7,43 ±1,51^{b}	7,71 ± 1,50^{b}	7,00 ± 1,00 ^{b}	0,001
Week 3 - Week 2	4,71 ± 0,76^{a}	7,00 ± 1,00^{b}	7,29 ± 1,25^{b}	7,71 ± 0,76 ^{b}	0,000
Week 4 - Week 3	2,14 ± 1,35^{a}	8,43 ± 0,79^{c}	4,86 ± 0,90^{b}	5,14 ± 1,35 ^{b}	0,000
Week 5 - Week 4	4,71 ± 1,11^{a}	8,57 ± 0,54^{b}	5,57 ± 1,13^{a}	5,43 ± 2,23 ^{a}	0,000
Week 6 - Week 5	5,57 ± 0,98^{a}	9,86 ± 0,90^{b}	6,43 ± 0,79^{a}	6,00 ± 1,00 ^{a}	0,000
Week 7 - Week 6	6,43 ± 1,72^{a}	9,00 ± 1,29^{b}	5,71 ± 1,60^{a}	5,29 ± 0,95 ^{a}	0,000
Average	18,86 ± 1,95^{a}	35,86 ± 1,35^{c}	22,57 ± 2,23^{b}	21,85 ± 1,57^{b}	0,000
P	0,000	0,000	0,001	0,000	

Table 4: The Average Difference in Weight Gain Sprague Dawley Rats during Research

Feed intake of the PC group was higher than the other groups. This is because of the provision of a high-fat diet high in carbohydrates in the PC group. The effect of high fructose, one of which will stimulate leptin resistance (Swarbrick et al., 2008), so that the body of the satiety signal is reduced. In addition, providing a high-fat diet would lead to insulin resistance (Park et al., 2001). This is comparable to the body weight of rats, where the PC group has a weight average highest compared to other groups.

3.4. Lipid Profile

The intervention of fermented red beans and steamed red beans can reduce cholesterol, triglycerides, and LDL levels and increase HDL levels significantly in the intervention group. In the RBF group, there was a decrease of 36.74% in total cholesterol, a 23.61% decrease in triglyceride levels, a 20.05% decrease in LDL levels, and a 14.85% increase in HDL. In the RBS group, there was a 51.18% decrease in total cholesterol, a 42.12% decrease in triglyceride levels, a 35.72% decrease IN LDL levels, and a 27.87% increase in HDL.

Group	Cholesterol total (mg/dl)		Triglyceride (mg/dl)		HDL (mg/dl)		LDL(mg/dl)	
	Pre	Post	Pre	Post	pre	Post	Pre	Post
NC	83,46±2,25^{a}	83,32±2,11^{a}	67,73±3,66^{b}	65,60±4,90^{d}	24,62±1,99^{a}	26,92±1,21^{a}	73,40±5,33^{a}	73,79±5,55^{a}
PC	189,14±4,16^{b}	190,01±4,67^{d}	25,46±1,51^{a}	24,48±1,67^{a}	76,32±1,36^{b}	77,28±1,56^{c}	129,13±2,44^{b}	130,42±2,26^{d}
RBF	188,55±3,20^{b}	119,29±3,30^{c}	25,07±0,91^{a}	39,92±2,11^{b}	76,32±1,98^{b}	56,27±1,66^{b}	127,01±1,81^{b}	97,02±2,74^{c}
RBS	187,87±3,16^{b}	91,71±4,41^{b}	24,39±2,02^{a}	52,26±1,50^{c}	75,13±1,86^{b}	39,41±1,89^{d}	126,50±3,02^{b}	84,38±1,84^{b}

Table 5: Lipid Profile Rats Serum before and after Intervention

3.5. Glucose Level

There is a significant difference ($p < 0.05$) in each group's average blood glucose levels between the pre-test and post-test. In the group of NC, blood glucose levels increased significantly to 0.78 mg /dl (1.18%) but were still within normal values. A significant increase also occurs in the PC group, which is equal to 0.60 mg/dl (0.3%). While the RBF group and RBS group decreased significantly. Glucose levels decreased in the TKM group by 58.08%, while in the SGA group, they were at 79.49% (Table 6).

Group	Glucose Level (mg/dl)	
	Pre	Post
NC	65.57+1.36^{a}	66.35+1.45^{a}
PC	167.72+2.83^{b}	168.32+2.84^{b}
RBF	168.92+5.15^{b}	106.86+2.82^{c}
RBS	167.18+2.25^{b}	93.14+2.31^{d}

Table 6: Glucose Level Rats Serum before and after Intervention

Red beans (Phaseolus vulgaris L.) are rich in dietary fiber, resistant starch, and saponins. Processing can affect bioactive compounds in red beans. Red beans fermented and red beans prevent weight gain through the mechanism of insoluble fiber and other components in them. Insoluble fiber can inhibit the absorption of lipids in the intestine through increased faecal mass in the digestive tract, reduced transit times, and increased laksasi (Cummings, 2001; Tosh, 2010). Soluble fiber, resistant starch, and phytohemagglutinin (PHA) have the same mechanism: regulating appetite and increasing satiety (Han et al., 2013; Han et al., 2005; Mansour et al., 2013). α-amylase inhibitor (α-AI) is a starch blocker that can lower glucose uptake and decrease the synthesis and storage of fat (Mansour et al., 2013). Saponins lose weight by inhibiting fat absorption (Putong, 2007), while catechin, anthocyanin, red beans, and quercetin can inhibit fat accumulation in the body, thereby preventing obesity.

Dietary fiber can improve the lipid profile and blood glucose (Marsono, 2008) by several mechanisms, among others fiber increases the excretion of bile acids (Insull, 2006), inhibits the enzyme 3-hydroxy 3-methyl glutaryl (HMG) CoA Reductase (Tala, 2009), and produces Short Chain Fatty Acid (SCFA) in the colon (Young & Le Leu, 2004).

The effects of resistant starch in improving the lipid profile and blood glucose lowering fiber are similar to the mechanism that inhibits HMG-CoA Reductase and produces SCFA. In addition, resistant starch can also increase the level of hepatic Scavenger Receptor Class B1 (SR-B1), which is the receptor that mediates the reception of HDL cholesterol to HDL cells. Starch resistance also increases cholesterol 7α-hydroxylation, which is an enzyme in the formation of bile acids (Han et al., 2003). Resistant starch forms a viscus or gel that can slow the absorption of nutrients including carbohydrates from the intestinal lumen, causing a decrease in free fatty acid production and secretion of the hormone insulin antagonist so that a sharp rise in blood glucose levels did not occur (Haralampu, 2000).

Saponins and LDL cholesterol levels with a mechanism to decrease the activity of HMG Co-A Reductase and increases the activity of 7α-hydroxylase (Alfrose et al., 2009). Saponins lower triglyceride levels by interfering with the formation of micelles so that the absorption of triglyceride food in the small intestine is reduced and inhibits pancreatic lipase (Han et al., 2000). Saponins can lower blood glucose by improving insulin response, increasing insulin signaling, increasing levels of plasma insulin and inducing the release of insulin from the pancreas, inhibiting the activity of disaccharide, activating glycogen synthesis, inhibiting gluconeogenesis, inhibiting the activity of alpha-glucosidase inhibition of mRNA expression of glycogen phosphorylase and glucose 6 phosphates, and increasing the expression of GLUT4 (insulin receptor in tissue) (Elekofehinti, 2015).

Red beans fermented and red beans steamed are equally effective in weight loss, improvements in lipid profiles, and blood glucose lowering. However, red beans steamed are more significant in weight loss, lipid profile improvements, and blood glucose lowering than red beans fermented. That is because the processing of red beans fermented takes longer, so they lose their nutritional value.

Excessive cooking with high temperatures will change the nutrient content in food ingredients (Purwaningsih, 2013). The boiling process in the manufacture of red beans fermented to reduce the content of nutrients due to dissolved in boiling water (Sundari et al., 2015). Based on research by Rosida et al. (2013), the processing affects resistant starch content. After the treatment with 24-hour steaming and then cooling, the starch resistance will increase by 103.9%, while the 24-hour boiling and cooling will increase starch resistance by 16.8%.

Based on research by Alajaji et al. (2006), the boiling process will reduce the content of saponins in most nuts (Cicer arietinum L.) in the other cooking process, namely 51.65%. In addition, the fermentation process will reduce and or eliminate the anti-nutritional substances, one of which is α-amylase inhibitor is a protein in red bean's role in lipid-lowering mechanism (Ramirez-Jimenez et al., 2015). The fermentation process will increase by 33.3% crude fiber, but the boiling process will lower crude fiber content by 36.1%. Therefore, the boiling process long before fermentation will reduce some of the nutritional value.

4. Conclusion

Giving red beans fermented and red beans steamed significantly reduces weight, improves lipid profile, and lowers blood glucose levels. Giving red beans steamed significantly more than the provision of red beans fermented. For the improvement of further research, need to be investigated bioactive compounds in soybean red beans and red beans steamed that play a role in weight loss, improvements in lipid profiles, and blood glucose as the content of dietary fiber, resistant starch, saponins, antioxidants, and others. This research also needs to be researched more about the acceptance of products and their effect on patients with metabolic syndrome.

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6. References

i. Afrose, S., Hossain, M.S., Maki, T. & Tsuji, H. (2009). Karaya Root Saponin Exerts a Hypocholesterolemic Response in Rats Fed a High-Cholesterol Diet. J Nutr Res., 29 (5):350–354.
ii. Alajaji, S.A. & El-Adawy, T.A. (2006). Nutritional Composition of Chickpea (*Cicer arietinum L.*) as Affected by Microwave Cooking and Other Traditional Cooking Methods. J Food Comp Analysis. 19:806–812.
iii. Astawan, M., Tutik, W. & Jefiaman, S. (2015). Pengaruh konsumsi tempe Kedelai Grobogan terhadap Profil Serum, Hematologi dan Antioksidan Tikus. J. Teknol. dan Industri Pangan., 26 (2): 155–162.
iv. Atchibri, A.L. Ocho-Anin, K.D. Brou, T.H. Kouakou, Y.J. & Kouakou, D.G. (2010). Screening for Anti-diabetic Activity and Phytochemical Constituents of Common Bean (Phaseolus vulgaris L.) seeds. J Med Plants Res., 4(17):1757–1761.
v. Cummings, J.H. (2001). The Effect of Dietary Fiber on Fecal Weight and Composition. In G. Spiller, & G. Spiller (Eds.), Dietary Fiber in Human Nutrition (pp. 183–252). Boca Raton, FL: CRC Press.
vi. Elekofehinti, O.O. (2015). Saponin: Anti-diabetic Principles from Medical Plants – A review. Pathophysiology, (22): 95–103.
vii. Gordon, D. T., Topp, K., Shi, Y.-C., Zallie, J. & Jeffcoat, R. (1997). Resistant starch: physical and physiological properties. In M. Yalpani (Ed.). New technologies for healthy foods & nutraceuticals (pp. 157–178)., ATL Press.
viii. Han, K., Fukushima, M., Shimizu, K., Kojima, M., Ohba, K., Tanaka, A., Shimada, K., Sekikawa, M. & Nakano, M. (2003). Resistant starches of beans reduce the serum cholesterol concentration in rats. J Nutr Sci Vitaminology., 49:281–286.

ix. Han, K., Iijuka, M., Shimada, K., Sekikawa, M., Kuramochi, K., Ohba, K., Ruvini, L., Chiji, H. & Fukushima, M. (2005). Adzuki Resistant Starch Lowered Serum Cholesterol and Hepatic 3-Hydroxy-3-Methylglutaryl- CoA mRNA Levels and Increased Hepatic LDL-Receptor and Cholesterol 7Ahydroxylase mRNA Levels in Rats Fed a Cholesterol Diet. British J Nutr., 94: 902–908.
x. Han, K.H., Fukushima, M., Kato, T., Kojima, M., Ohba, K., Shimada, K.I., Sekikawa, M. & Nakato, M. (2003). Enzyme-Resistant Fraction of Beans Lowered Serum Cholesterol and Increased Sterol Excretions and Hepatiz mRNA Levels in Rats. Lipids. 38 (9): 919–924.
xi. Han, L.K., Xu, B.J., Kimura, Y., Zheng, Y.N. & Okuda, H. (2000). Platycodi Radix Affects Lipid Metabolism in Mice With High Fat Diet Induce Obesity. J Nutr. 130: 2760–2764.
xii. Haralampu, S.G. (2000). Resistant Starch - A Review of the Physical Properties and Biological Impact of RS3. Carbohydrate Polymer, 41:285–292.
xiii. Insull, W. (2006). Clinical Utility of Bile Acid Sequestrants in the Treatment of Dyslipidemia: A Scientific Review. Southern Med J., 99 (3): 257–273.
xiv. International Diabetes Federation. 2006. The Metabolic Syndrome. IDF Consensus.
xv. Kemenkes. (2007). *Riset Kesehatan Dasar 2007*. Kementrian Kesehatan Republik Indonesia.
xvi. Kemenkes. (2013). *Riset Kesehatan Dasar 2013*. Kementrian Kesehatan Republik Indonesia.
xvii. Mansour, A., Hosseini, S., Larijani, B., Pajouhi, M. & Mohajeri-Tehrani, M. (2013). Nutrition Related to GLP-1 Secretory Responses. Elsevier Nutr. (29): 813–820.
xviii. Marsono, Y., Wiyono, P. & Noor. Z. (2002). Indeks Glikemik Kacang-kacangan. Jurnal Teknologi dan Industri Pangan Vol. XIII no 3.
xix. Marsono, Y, Zuheid N. & Fitri R. (2003). Pengaruh Diet Kacang Merah terhadap Kadar Gula Darah Tikus Diabetik Induksi Alloxan. Jurnal Teknologi dan Industri Pangan Vol. XIV, no 1.
xx. Marsono, Y. (2008). Prospek Pengembangan Makanan Fungsional. Jurnal Teknologi Pangan dan Gizi. 7 (1) : 19–27.
xxi. Park, S., Kim, Y.W., Jang, K. & Lee, S.K. (2001). Effect of High Fat Diet on Insulin Resistance: Dietary Fat Versus Visceral Fat Mass. J Korean Med Sci., 46 (12); 1594–1603.
xxii. Purwaningsih, S., Salamah, E. & Apriyana, G.P. (2013). Profil Protein dan Asam Amino Keong Ipong-ipong (*Fasciolaria salmo*) pada Pengolahan yang Berbeda. Jurnal Gizi dan Pangan. 8(1): 77–82.
xxiii. Putong, O.T. (2007). Pengaruh Pelarut Ekstraksi Daun Jati Belanda (Guazuma ulmifolia L.) Terhadap Penurunan Nafsu Makan dan Berat Badan Tikus Putih Jantan dengan Uji Leptin. Skripsi. Widya Mandala.
xxiv. Ramirez-Jimenez, A.K., Reynoso-Camacho, R., Terejo, M.E., Leon-Galvan, F. & Loarca-Pina, G. (2015). Potential Role of Bioactive Compounds of *Phaseolus vulgaris L*. On Lipid-Lowering Mechanisms. J Food Res Internat., 76: 92–104.
xxv. Reeves, Philip G., Nielsen, F.H., Fahey, G.C. (1993). AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. J. Nutr. (123): 1939–1951.
xxvi. Rosida. & Yulistiani, R. (2013). Pengaruh Proses Pengolahan terhadap Kadar Pati Resisten Sukun (Artacarpus altilis Park). Universitas Pembangunan Negeri Veteran Jawa Timur.
xxvii. Setiawan, B. & Eko S. (2005). Stres Oksidatif dan Peran Antioksidan pada Diabetes Mellitus. Majalah Kedokteran Indonesia, Vl: 55, No: 2
xxviii. Sundari, D., Almasyhuri. & Lamid, A. (2015). Pengaruh Proses Pemasakan Terhadap Komposisi Zat Gizi Bahan Pangan Sumber Protein. Media Litbangkes, 25 (4):235–242.
xxix. Swarbrick, M.M., Stanhope, K., Elliott, S.S., Graham, J.L., Kaus, R.M. & Christiansen, M.P. (2008). Consumption of Fructose Sweetened Beverages for 10 Weeks Increase Postprandial Tryglicerol and Apolipoprotein-B Concentration in Overweight and Obese Women. British J Nutr. 29:F625–F631.
xxx. Tala, Z. (2009). Manfaat Serat Bagi Kesehatan. Universitas Sumatera Utara.
xxxi. Tosh, S. & Yada, S. (2010). Dietary Fibres in Pulse Seeds and Fractions: Characterization, Functional Attributes, and Applications. Food Res Internat., 43, 450–460.
xxxii. Utari, Diah M., Rimbawan, Hadi Riyadi, Muhilal & Purwantyastuti. (2011). Potensi Asam Amino pada Tempe untuk Memperbaiki Profil Lipid dan Diabetes Melitus. Jurnal Kesehatan Masyarakat Nasional, 5 (4).
xxxiii. WHO. (1999). The world health report 1999 – Making a Difference. World Health Organization.
xxxiv. WHO. (2011).Global Status Report on Non-communicable Disease 2010. World Health Organization.
xxxv. Young, G.P. & Le Leu, R.K. (2004). Resistant Starch and Colorectal Neoplasia. Journal of AOAC International., 87 (3): 775–786.