Jurnal Riset Kesehatan, 13 (1), 2024, 44 - 54

DOI: 10.31983/jrk.v13i1.11640

Jurnal Riset Kesehatan

http://ejournal.poltekkes-smg.ac.id/ojs/index.php/jrk

TEMPE GEMBUS INTERVENTION DECREASED TOTAL CHOLESTEROL AND TRIGLYCERIDE IN OBESE WOMEN

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Abstract

Obesity increases cardiovascular risk through dyslipidaemia. High fibre diet reduces risk factors of dyslipidaemia by up to 50%. Tempe gembus is a solid fermented food made from tofu waste product with rhyzopus-oligosporus, that contained high fibre. The purpose of this study is to determine the effect of the administration processed tempe gembus on lipid profiles in obese women. This study was pre-post randomized control group design. Forty-four pre-menopausal women with obesity, dyslipidaemia took part in this study, divided into 2 groups, includes the intervention group and the control group. The Intervention group received 150 gram of processed tempe gembus for 28 days. Statistical analysis independent sample test or Mann Witney were used to analyse differences pre and post-lipid profile between intervention and control group. Consumption of 150 g/day of processed tempe gembus for 28 days reduced total cholesterol levels by 13.4 mg/dl (p = 0.012), HDL cholesterol by 2.8 mg/dl (p = 0.082), LDL cholesterol by 17.6 mg/dl (p = 0.52) and triglyceride levels of 5.3 mg/dl (p = 0.05), respectively. There was significant effect of processed tempe gembus consumption to reduce total cholesterol, and triglycerides. Unfortunately, there were no effect on LDL and HDL cholesterol.

Keywords: *Tempe gembus;cholesterol;LDL;HDL;triglycerides;obesity*

1. Introduction

Obesity is a pathological condition characterized by fat accumulation in adipose tissue that is harmful to people health. The prevalence of obesity has increased throughout the world, including Indonesia. The incidence of obesity in Indonesian women tends to be higher when compared to men. Study Cross Sectional of Health Study in 2018 showed that the prevalence of obesity in adult men was 14.5%, whereas in women it was 29,3% (Kemenkes, 2018)(Kemenkes RI, 2013). The prevalence of obesity in Central Java in adult women has also increased from 27,5 in 2013 to 30% in 2018 (Kemenkes, 2018):(Kesehatan et al., n.d.). As many as 74% female subjects in Class II Penitentiary Semarang were obese (Silfiya et al., 2016).

Obesity have been shown to be associated with several conditions such as diabetes mellitus, cardiovascular disease, hypertension, and dyslipidaemia. Obesity increases cardiovascular risk through risk factors such as increased the levels of triglycerides, *low density lipoprotein* (LDL), total cholesterol and decreased levels of *high density lipoprotein* (HDL) (Vekic et al., 2019).

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Food intake and dietary patterns play important role for the prevention and management of

DOI: 10.31983/jrk.v13i1.11640

cardiovascular disease (Anand et al., 2016). High-fibre diet, that is contains ≥ 25 grams of soluble fiber and ≥ 47 grams of insoluble fibre per day can reduce the risk of up to 50% of stroke and decreased risk of cardiovascular diseases (Casiglia et al., 2013; Fu et al., 2022). People in Indonesia having less fiber intake < 5 portion per days, that was 93,5% in 2013 and increased 95,5% in 2018 (Ministry and Health RI, 2018). One of high fibre food is tempe gembus. Tempe gembus is Indonesian authentic food, made from tofu wasted product, fermented with *Rhizopus oligosporus*. Tempe gembus is widely consumed as snack or side dish, in Javanese people. The fibre content in tempe gembus is about 10 gram/100 grams, 3 times higher than soybean tempe. Giving *tempe gembus* at a dose of 8% and 12% for 5 weeks in animals study showed a decrease in total cholesterol, *low density lipoprotein* (LDL) cholesterol and an increase in *high density lipoprotein* (HDL) (Sulchan & Rukmi, 2007).

High-fiber diets work by binding bile acids, increasing cholesterol excretion and forcing the liver to use cholesterol for the synthesis of new bile acids, which lowers blood cholesterol levels. Furthermore, the bacteria in the colon that produce fermented fiber stop the liver from synthesizing cholesterol. Intestinal bacteria transform the fermented food fiber into short-chain fatty acids, which can reduce blood cholesterol (Reynolds et al., 2022; Soliman, 2019).

The purpose of this study is to determine the effect of giving processed tempe gembus on lipid profiles in obese women.

2. Method

Study Design and Setting

This was an experimental study with *pre-post randomized control group design*, involving 40 *pre-menopausal women* in Class II Women's Penitentiary in Semarang City. Subjects were those who met the inclusion criteria, including the age of 20-50 years, premenopausal, non-pregnant and obese (BMI level ≥ 25 kg /m^2, or waist circumference ≥ 80 cm), dyslipidaemia (total cholesterol> 200 mg /dl, or LDL > 130 mg / dl, or triglycerides > 150 mg / dl, or HDL <40 mg / dl), and gave valid written consent after receiving further explanation. Women prisoners who were not willing to participate, and those having a history or experiencing of liver disease, kidney disease, thyroid disorders, cancer, coronary heart disease, stroke, diabetes mellitus, smoking in the last 3 months, taking nutritional supplements regularly, on hypoglycemic medication, phytopharmaca, hypolipids, and vegetarians were excluded from this study.

Interventions

Material of this study sample used tempe gembus which is made in laboratorium of food technology Poltekkes Kemenkes Semarang. Tempe gembus used was from tofu residue in manufacturer Cinde-Lamper Semarang. The control group was given a 1500 kcal/day diet, while the treatment group was given processed tempe gembus as much as 150/day for 28 days into the 1500 kcal/day diet given at lunch, for consumption at lunch and afternoon snacks. Processed tempe gembus that were given consists of 5 kinds of dishes namely stir-fried tempe gembus, *bacem, balado, pepes, and satay*. Subject in treatment group were given education for increasing consumption of tempe gembus optimally, and was given of fruit as much as 100 grams per days.

The obesity index used in this study was the body mass index and waist circumference. The body mass index was calculated as weight (kg)/(height x height) (m2). Waist circumference was measured using met-line. Measurement of lipid profile levels using a lipid profile examination tool. Arterial Blood Pressure was measured on the right arm using Omron Digital Tensimeter. Assessment of food intake using forms *food recall* 24-hour. Assessment of

DOI: 10.31983/jrk.v13i1.11640

the subject's compliance in consuming tempe gembus using the monitoring form and food consumption form. Assessment of physical activity using the form *Baecke* activity.

All variable analysed descriptively as means and standard deviations, medians, minimum and maximum values. Normality test used was the Shapiro Wilk test. The differences of baseline characteristic including protein, fat, carbohydrate intake and food intake during intervention (protein, carbohydrate, cholesterol, and fibre) between two group was analysed using independent sample test because the data were normal distribution. Mann Whitney test is used to analyse differences of age, body weight, BMI, waist circumference, systolic and diastolic BP, physical activity, energy intake before intervention, food intake during intervention (energy and fat) between control and intervention group.

The difference between the group of lipid profile levels including total cholesterol (before, after and delta), HDL-C (after and delta), and LDL-C (before, after, and delta) was analysed using independent sample test. The data of lipid profile levels including HDL-C before intervention and Tryglicerides level (before, after and delta) were not normal distribution so the analyse used Mann Whitney test. Paired sample test is used to analysed the difference before and after intervention of total cholesterol in two group, HDL-C in intervention group, and LDL-C in two group. Wilcoxon test is used to analysed the difference before and after intervention of HDL-C in control group, and tryglicerides in two group.

3. Result and Discussion

Subject characteristics

Table 1. Baseline Characteristics of Subjects

Characteristic	Group		*P*
	Control (n=20)	Intervention (n=20)	
	Mean± SD	Mean± SD	
Age (years)	35.1 ±8.54	36.5 ± 9.38	0.645[b]
Body Weight (kg)	72.1 ± 15.25	66.2 ± 6.97	0.323[b]
BMI (kg/m2)	29.9 ± 5.62	28.0 ± 2.60	0.433[b]
Waist Circumference (cm)	97.7 ± 10.13	90.2 ± 6.36	**0.022[b]**
Systolic BP (mmHg)	112.8 ± 15.45	121.9 ± 26.98	0.628[b]
Diastolic BP (mmHg)	80.1 ± 10.08	81.3 ± 13.36	0.460[b]
Physical activity	1.5 ± 0.18	1.5 ± 0.12	0.881 [b]
Energy Intake (kcal/day)	2082.5 ± 310.59	1976.5 ± 266.57	0.224[b]
Protein Intake (g/day)	89.6 ± 23.68	80.0 ± 23.85	0.211[a]
Fat Intake (g/day)	66.2 ± 11.11	64.7 ± 16.88	0.744[a]
Carbohydrate Intake (g/day)	240.1 ± 78.31	244.0 ± 48.23	0.852[a]

[a] *Independent Sample test*
[b] *Mann Whitney test*

Table 1 presents the characteristics of the research subjects before the study for each group. Subjects age ranged between 20 to 50 years. There were no differences of baseline characteristics (including age, body weight, BMI, systolic blood pressure, diastolic blood pressure and physical activity) between control and intervention group, considering both groups were equal (p values> 0.05). Anthropometric data of waist circumference showed a statistically significant difference with p value <0.05. Therefore, waist circumference remained as confounding variable.

Nutrient Intake During Intervention Period

Table 2 presents the data on food intake during the study in each group. Meal intake during the study in the treatment group was calculated from the intake of prisons fed with the addition of processed tempe gembus 150 grams / day, whereas in the control group only counted from foods that were given by prisons authority.

DOI: 10.31983/jrk.v13i1.11640

Table 2. Nutrient Intake During Intervention

Indicators	Group		*P*
	Control Mean ± SD	Intervention Mean ± SD	
Energy Intake (kcal/day)	1598,1± 286,99	1769,8 ± 370,92	0,137[b]
Protein Intake (g/day)	59,1 ± 13,47	66,9 ± 14,44	0,085[a]
Fat Intake (g/day)	54,1 ± 17,77	58,5 ± 13,45	0,160[b]
Carbohydrate Intake (g/day)	221,4 ± 31,92	249,7 ± 58,10	0,064[a]
Cholesterol Intake (mg/day)	198,9 ± 91,82	240,9 ± 78,81	0,129[a]
Fiber Intake (g/day)	10,4 ± 2,47	20,8 ± 3,94	0,000[a]

[a] *Independent Sample test*
[b] *Mann Whitney test*

The intake of energy, fat, protein, carbohydrates, cholesterol and fibrr in the control group was lower than the treatment group. Based on the adequacy of nutrition in both groups given a 1500 kcal / day diet has over. Statistically, food intake during the intervention (energy, fat, protein, carbohydrate and cholesterol) in each control group and treatment group did not show any significant difference with p value> 0.05.

Cholesterol intake in the control group had complied with the NCEP ATP III recommendation of less than 200 mg / day, whereas in the treatment group the average cholesterol intake was more than 200 mg / day. However, each individual has a varied cholesterol response to convert cholesterol to bile acids.

The average fibre intake in the treatment group was in accordance with the NCEP ATP III recommendation of 20-30 g / day. The fibre intake during the intervention showed a significant difference between the control group and the treatment group. Therefore, fibre intake during the study was included in the confounding variable. The mechanism of high fibre intake by reducing blood cholesterol levels is by binding to bile acids which can increase cholesterol excretion.

Table 3. Changes in Lipid Profile (Total, HDL, LDL cholesterol, Trygliceride Levels) Before and After Intervention

Lipid Profile	Control (n=20) Mean ± SD	Intervention (n=20) Mean± SD	p
Cholesterol Level			
Before	204,8 ± 34,09	212,2 ± 20,95	0,411[a]
After	203,0 ± 31,89	198,9 ± 24,33	0,646[a]
Δ Cholesterol	-1,8 ± 15,31	-13,4 ± 20,49	0,012[a]
P	0,615[c]	0,009[c]	
HDL cholesterol			
Before	49,5 ± 9,65	52,0 ± 10,38	0,327[b]
After	44,7 ± 6,69	49,3 ± 71,36	0,064[a]
Δ HDL	-4,4 ± 6,74	-2,8 ± 7,77	0,082[a]
P	0,002[d]	0,130[c]	
LDL cholesterol			
Before	140,1 ± 21,18	143,7 ± 29,75	0,653[a]
After	129,2 ± 18,71	126,2 ± 26,68	0,678[a]
Δ LDL	-10,8 ± 18,40	-17,6 ± 42,67	0,520[a]
P	0,016[c]	0,081[c]	
Triglyceride			
Before	116,9 ± 53,03	138,7 ± 43,51	0,110[b]
After	145,9 ± 50,75	133,4 ± 89,81	0,102[b]
Δ Triglyceride	29,0 ± 80,97	-5,3 ± 106,05	0,050[b]
P	0,103[d]	0,279[d]	

[a] *Independent Sample test,* [b] *Mann Whitney test,*
[c]*Paired Sample test*, [d]*Wilcoxon test*

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Total cholesterol levels before the intervention 204.8 ± 34.09 mg / dl decreased to 203.0 ± 31.89 mg / dl after intervention, whereas the decrease in total cholesterol levels in the control group was 1.8 ± 15.31 mg / dl (p> 0.05). The decrease in total cholesterol levels in the treatment group was -13.4 ± 20.49 (p <0.05). While the change in total cholesterol levels there was a significant difference between the control group and the treatment group (p <0.05). This is in line with research conducted by M. Sulchan and MG Isworo Rukmi in hyperlipidemic mice showing that administration of tempe gembus 8% and 12% per 100 grams can change serum lipid profile, by reducing total cholesterol (Sulchan & Rukmi, 2007).

In the control group, HDL cholesterol levels before the intervention of 49.5 ± 9.65 mg / dL dropped to 44.7 ± 6.69 mg / dl after intervention. The decrease in HDL cholesterol levels in the control group (4.4 ± 6.74 mg / dl, p <0.05) was higher than in the treatment group (2.8 ± 7.77 mg / dl, p> 0.05) after intervention. This study is in line with a quasi-experimental study on 45 women in the Semarang Mayor's office which shows that there is no significant difference in the increase in HDL cholesterol levels significantly (Nabilah, 2016). The results of this study are in line with those of research by Kuntantarti, et al., Showing that there is a decrease in HDL cholesterol levels but not significantly. Treatment performed does not affect HDL cholesterol levels and maintain that HDL levels tend to remain (Kuntarti et al., 2019).

A decrease in LDL cholesterol levels in both groups. LDL cholesterol levels in the control group from 140.1 ± 21.18 mg / dl to 129.2 ± 18.71 mg / dl after the intervention, whereas in the treatment group it fell by 17.6 ± 42.67 mg / dl (p> 0.05). The difference in decreasing LDL cholesterol levels in the treatment group did not differ statistically compared to the control group. This study is not in line with a quasi-experimental study on 45 women in the Semarang Mayor's office which shows that there are differences in the mean reduction in LDL cholesterol levels by administering tempe gembus (Nabilah, 2016).

In the control group, triglyceride levels before the intervention were 116.9 ± 53.03 mg / dl to 145.9 ± 50.75 mg / dl after intervention. An increase in triglyceride levels in the group of 29.0 ± 80.97 mg / dl (p> 0.05). Whereas in the treatment group, triglyceride levels decreased by 5.3 ± 106.05 mg / dl (p = 0.05). There were significant differences in triglyceride levels in the control group and the treatment group (p = 0.05). Giving tempe gembus intervention influences triglyceride levels.

Effect of Treatment on Lipid Profile

Cholesterol

The effect of treatment on total cholesterol reduction was tested using Anova Repeated Measure controlled by fiber intake, cholesterol, physical activity and decreased BB, as shown in the figure 1.

DOI: 10.31983/jrk.v13i1.11640

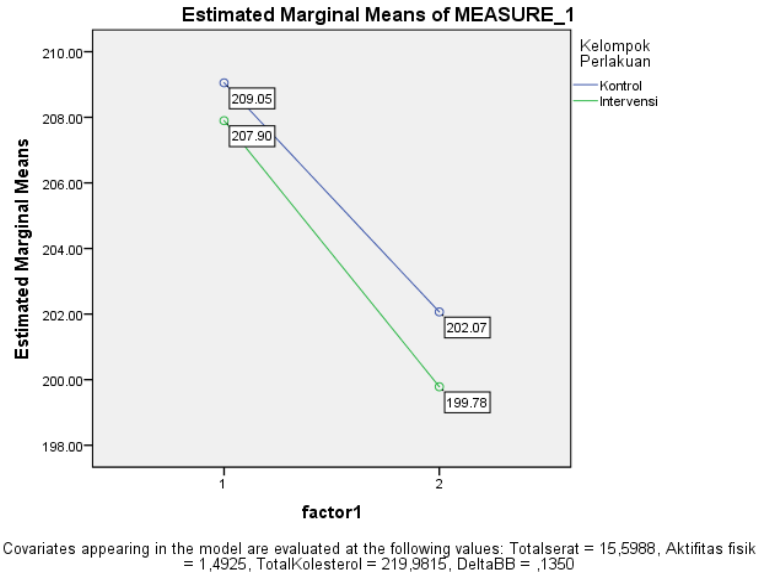

Figure 1. *The effect of tempe gembus on cholesterol*

Total cholesterol decreased from 207.9 to 199.78 mg / dl in intervention group (figure 1.). The decrease in total cholesterol was not significant, resulting in an interaction between changes in total cholesterol levels that were not significant after being treated for 28 days (p = 0.920). Decreasing total cholesterol levels occurs due to the increased fibre intake in the treatment group by giving consumption of tempe gembus as much as 150 g / day for 28 days. Food fibre contained in tempe gembus 3 times greater than fibre in soybean tempe (Sulchan & Rukmi, 2007). Fibre in the digestive tract will bind bile acids and then excreted through faeces. Increased excretion of cholesterol in the stool will reduce cholesterol levels that go to the liver, thereby increasing the uptake of cholesterol in the blood that is synthesized to become bile acids. Therefore, cholesterol levels in the blood plasma decrease. Fibre consumption can reduce the YY peptide hormone which plays a role in regulating appetite by slowing the time of emptying the stomach so that it can increase satiety ((Finelli et al., 2016; Waddell & Orfila, 2023)

In addition, tempe gembus has high antioxidants. The antioxidant compound in tempe gembus is isoflavone. The results of this study concur with those of Bintanah et al. which is a significant relationship between isoflavone-rich food intake and total blood cholesterol levels (Bintanah et al., 2017). The mechanism of isoflavone reduces cholesterol, namely the occurrence of fat cell catabolism to produce energy, reduce LDL levels in the blood and reduce blood cholesterol levels.

Cholesterol HDL

The effect of treatment on reducing HDL cholesterol is tested using Anova Repeated Measure which is controlled by fibre intake, cholesterol, physical activity and decreased body weight, age, and waist circumference as shown in Figure 2.

DOI: 10.31983/jrk.v13i1.11640

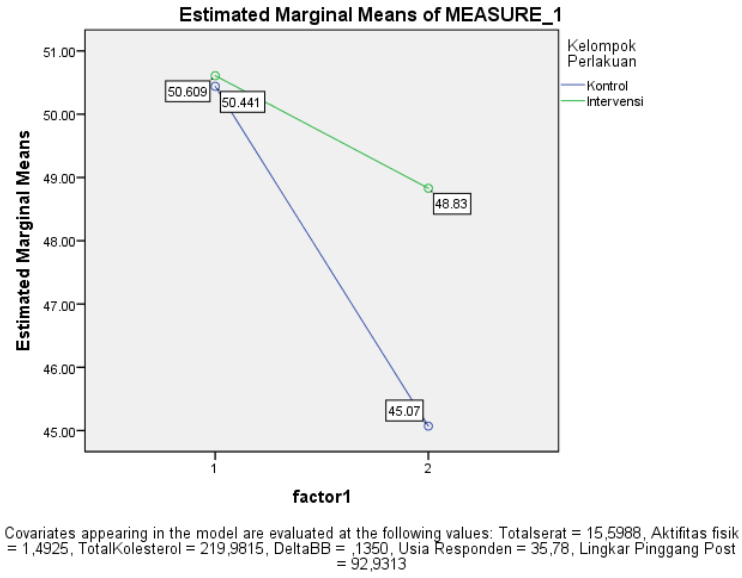

Figure 2. *The effect of tempe gembus on HDL-C Level*

The figure 2. shows that both in the control and intervention group experienced a decrease in HDL cholesterol levels. This results in line with a study suggested that soluble fiber supplementation had no signi ficant effect in HDL cholesterol compared with placebo . The decrease of HDL cholesterol in intervention was smaller compared to the control group. The interaction effect of treatment of tempe gembus for 28 days on HDL cholesterol levels was not significant (p = 0.714).

The difference in the occurrence of decreased HDL cholesterol levels in this study was caused by fibre intake in tempe gembus. Fibre intake can significantly increase HDL cholesterol levels (Anderson et al., 2009). The administration of fibre in hyperlipidaemic patients by 2.7 g/day for 20 weeks can increase HDL cholesterol levels by 3.9 mg/dl (W et al., 2008). Similar research also mentions that the administration of 4.8 g and 9.6 g of fibre for 14 days increase HDL cholesterol levels (Ikhsan & Mulyati, 2013). The average fibre intake contributed by the tempe gembus in this study was 9.7 grams. The results of a meta-analysis study from a randomized controlled trial showed that fibre intake had no significant effect on the increase in HDL cholesterol (Yanai & Tada, 2018).

Cholesterol LDL

The effect of the treatment on reducing LDL cholesterol is tested using Annova Repeated Measure which is controlled by the intake of fibre, cholesterol, fat, physical activity, age, and waist circumference.

DOI: 10.31983/jrk.v13i1.11640

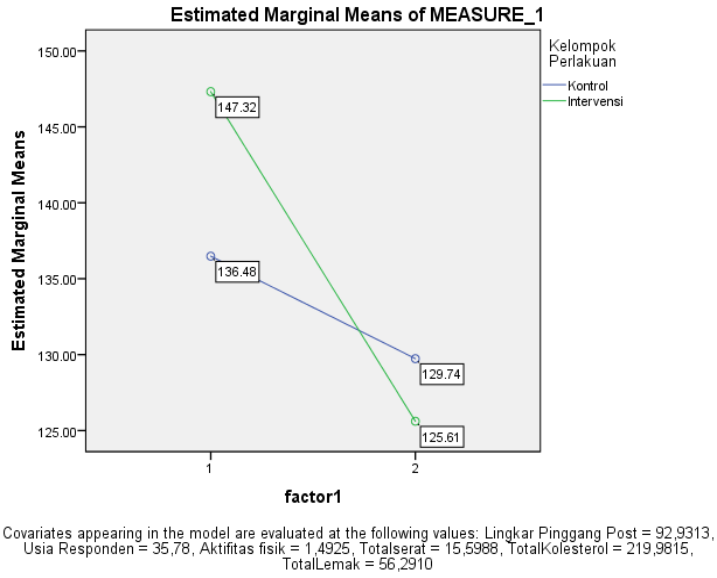

Figure 3. *The effect of tempe gembus on LDL-C level*

Figure 3. shows that the intervention group decrease in LDL cholesterol levels from 147.32 to 125.61. Whereas in control group, LDL cholesterol levels decreased from 136.48 to 129.74. The interaction effect of treatment for 28 days on LDL cholesterol levels was not significant (p = 0.780). It can be concluded that the treatment has not been able to significantly reduce LDL cholesterol levels.

Free radicals oxidize LDL cholesterrol, inflame cells and increase the risk of heart disease. Antioxidants can play a role against free radicals. Tempe gembus contains antioxidants in the form of high isoflavones. Isoflavones in tempe gembus can improve lipid profile in the body (E & FM, 2011). The mechanism of isoflavone decreases LDL levels associated with *sterol regulatory element binding protein* (SREBP), as a result LDL receptor gene excretion occurs and binds to estrogen receptors and becomes a ligand of *peroxisome-proliferator activated receptor* (PPAR). Cholesterol will be taken out of macrophages and small intestine and will be converted into bile salts (Kuntarti et al., 2019).

In addition, the content of tempe gembus which plays a role in improving lipid profile is amino acids (Utari et al., 2011). The composition of amino acids in tempe gembus is almost the same as soybean tempe, but in the tempe gembus there is no detection of proline, cysteine and tryptophan. Amino acids in vegetable proteins tend to reduce cholesterol levels, this is in contrast to amino acids contained in animal proteins that are hypercholesterolemia. Soy protein will reduce the absorption of cholesterol in the intestine and will increase the secretion of bile acids. Previous studies suggest that consumption of 25 grams to 50 grams of soy protein per day effectively reduces LDL by 4 - 8% and improves lipid profile(Utari et al., 2011).`

Therefore, LDL cholesterol levels in the treatment group experienced a greater decrease compared to the group that was not treated because of the addition of amino acids through the *tempe gembus* given for 28 days. The decrease in LDL levels showed no significant difference between the control and treatment groups, this could be due to a diet that was not strictly adhered to and strictly controlled, indicated by a higher intake of cholesterol and fat in the treatment group.

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Trygliceride Level

Trygliceride levels in the blood are affected by carbohydrate intake, PUFA, physical activity, decreased body weight, age, waist circumference and blood glucose. The effect of administration of processed tempe gembus on triglyceride levels that have been controlled by these factors can be seen in Figure 4.

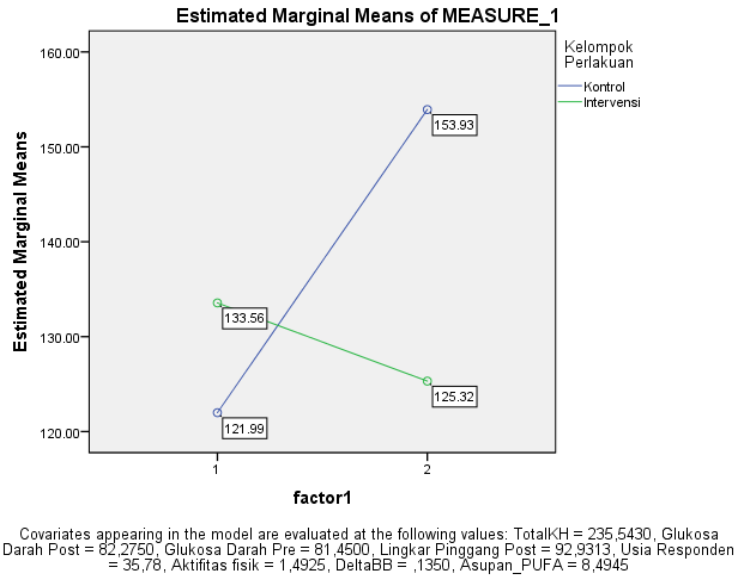

Figure 4. *The effect of tempe gembus on trygliceride level*

Picture above shows that the group given the treatment given tempe gembus decreased triglyceride levels from 133.56 mg / dl to 125.32 mg / dl. Conversely in the control group triglyceride levels increased from 121.99 mg / dl to 153.93 mg / dl. However, statistically the interaction effect of the administration of processed tempe gembus for 28 days which was controlled by factors of carbohydrate intake, PUFA, physical activity, weight reduction, age, waist circumference and blood glucose on triglyceride levels was not statistically significant (p = 0.601).

This is consistent with experimental analytic studies on 18 white male wistar rats which showed that there was an effect of giving tempe gembus to triglyceride levels that received a high cholesterol diet (p <0.05) (Tarisi et al., 2014). Other studies have shown that administration of tempe juice can reduce total cholesterol and triglyceride levels in outpatient hypercholesterolemia patients at Avicenna Bireuen Hospital (Mulyani & Rafiqa, 2018). Research with the treatment of soybean milk dose of 9 g / kg BW for 56 days can reduce total cholesterol and triglyceride levels (Nurcahyaningtyas, 2012).

4. Conclusion and Suggestion

Administration 150 gram of processed tempe gembus reduced total cholesterol and triglyceride levels in pre-menopausal obese women.

DOI: 10.31983/jrk.v13i1.11640

5. Acknowledgments

We thank to the Chief and staffs of Class II Women's Penitentiary in Semarang City which has provided permit and help during the research. The authors received financial support for the research by DIPA Health Polytechnic of Semarang with number of grant : PT-4-1-079

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