

## EFFECTS OF EDAMAME (*Glycine max*) EXTRACT ON POST-PRANDIAL SERUM TRIGLYCERIDE IN WISTAR RATS

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### ABSTRAK

*Edamame (Glycine max) adalah sediaan kacang kedelai yang belum dewasa dan masih dalam polongnya, dimana edamame sendiri memiliki kadar kalsium dan serat yang tinggi, dua bahan aktif yang dijelaskan dalam penelitian-penelitian sebelumnya dapat mempengaruhi kadar serum trigliserida post-prandial. Tujuan dari penelitian ini adalah untuk menganalisis efek dari ekstrak edamame (Glycine max) pada kadar serum trigliserida tikus setelah administrasi minyak kelapa sawit melalui jalur intragastrik. Penelitian ini menggunakan minyak kelapa sawit, ekstrak edamame (Glycine max), dan eter. Desain penelitian ini adalah experimental post-test, dimana desain ini bertujuan untuk mengukur kadar trigliserida serum setelah pemberian minyak kelapa sawit. Trigliserida diukur pada tiap subjek dua kali, pertama setelah pemberian minyak kelapa sawit tanpa ekstrak edamame dan kedua setelah pemberian minyak kelapa sawit dengan ekstrak edamame. Terdapat periode washout selama 1 minggu antara dua waktu saat sampel diambil. Hasil tiap subjek kemudian dibandingkan. Kadar trigliserida serum post-prandial 2 jam setelah pemberian minyak kelapa sawit tanpa dan dengan ekstrak edamame pada tikus tidak menunjukkan perbedaan yang berarti. Sebagai simpulan, ekstrak edamame tidak memiliki efek pada trigliserida serum post-prandial 2 jam setelah pemberian minyak kelapa sawit jalur intragastrik. (FMI 2016;52:57-61)*

**Kata kunci:** Edamame, minyak kelapa sawit, trigliserida serum, post-prandial, tikus Wistar

### ABSTRACT

*Edamame (Glycine max) is a preparation of immature soybeans in the pod, which is high in calcium and dietary fiber, two elements described in previous studies that could alter the level of post-prandial serum triglyceride. The purpose of this research is to analyze the effect of edamame (Glycine max) extract on post-prandial serum triglyceride in rats after intragastric administration of palm oil. The materials used in this study are palm oil, edamame extract, and ether. The design of this study is experimental post-test study design, which is a design to measure the serum triglyceride level after meal with and without Glycine max. Triglyceride is measured on each subject two times on total, first one after consumption of meal without Glycine max and the second one after consumption of meal with Glycine max. There is a washout period of 1 week between two times the samples were taken. Both results are then compared in every subject. The level of 2-hour post-prandial serum triglyceride in rats after palm oil without intragastric edamame (Glycine max) extract administration and with intragastric edamame (Glycine max) extract administration showed no significant difference. In conclusion, edamame (Glycine max) extract had no effect on 2-hour post-prandial serum triglyceride after palm oil administration via intragastric tube. (FMI 2016;52:57-61)*

**Keywords:** Edamame, palm oil, serum triglyceride, post-prandial, Wistar rats

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### INTRODUCTION

Obesity is not just a cosmetic problem. This condition also greatly increases the risk of many diseases. These diseases include coronary heart disease, high blood pressure, stroke, type 2 diabetes mellitus, metabolic syndrome, cancer, osteoarthritis, sleep apnea, hypoventilation syndrome, reproductive problems, etc (National Heart, Lung, and Blood Institute 2012). During the year 2013, the prevalence of obesity in adult females in Indonesia (>18 years old) was 32.9%, showing an 18% increase compared to 2007 (13.9%)

and 17.5% compared to 2010 (15.5%). In adult males (>18 years old), the prevalence of obesity during year 2013 was 19.7%, higher compared to 2007 (13.9%) and 2010 (7.8%) (Report of Basic Health Research (Risesdas) 2013).

Basically, it is caused by the intake of calories that exceeds the calories spent. The causes include poor diet (especially the fatty and sugary ones), lack of physical activity, genetics, and medical reasons such as hypothyroidism and Cushing's syndrome (National Health Services UK 2014). Modern lifestyle influences the

physical activity of people. Over time, humans created technology and inventions that eases daily activities, some through reduction of movements and mobilization. In Indonesia, cheap high caloric snacks are sold throughout the streets, especially those rich in sugar and fat. These two combined, both lack of physical activity and high caloric intake could be the contributing factors of the increase of obesity prevalence in Indonesia.

It was proven in a study that calcium intake has negative relations with obesity-related disorders (Jacqmain et al 2003). In that particular observational study, it was concluded that low calcium intake is associated with greater adiposity, particularly in women. Several studies have also shown that dairy calcium has a negative relation with post-prandial lipid response, specifically on chylomicron triacylglycerol (Lorenzen et al 2007), and could reduce adipocyte mass through disinhibition of lipolysis (Zemel et al 2000). Although certain researches have shown that there are beneficial factors of calcium for lipid metabolism, none of those have really proven the beneficial factor of non-dairy calcium for lipid metabolism.

Dietary fiber has also been long known for its long-term beneficial effects on lipid metabolism, but not very clearly explained on how it affects fat digestion. It was then explained that results in animal studies suggests that the presence of fiber-rich diet could reduce or delayed and intestinal absorption of fat and cholesterol could be impaired (Cara et al 1992). Edamame (*Glycine max*) is a preparation of immature soybeans in the pod, commonly found in the cuisine of Taiwan, China, Japan, Indonesia, Korea, and Hawaii. It serves as an appetizer or side dish. It is said to have been eaten in China and Japan for thousands of years. Edamame has a high content of both calcium and dietary fiber.

A hundred grams of edamame contains 70.0 – 72.0 miligrams of calcium and 13.8-15.6 grams of dietary fiber (Johnson et al 1999). It could be speculated that with high contents of both, calcium and dietary fiber, edamame could alter the lipid metabolism in the intestines, specifically by making lipid absorption more ineffective leading to a lower post-prandial serum triglyceride. The aim of this study is to analyze the effect of edamame (*Glycine max*) on serum triglyceride level, which is a type of lipid that attributes to the amount of energy in a diet, after palm oil administration via intragastric tube in Wistar rats.

## MATERIALS AND METHODS

The materials used in this experiment are palm oil, edamame extract, and ether. The palm oil used is

branded Bimoli. The edamame used for extraction is bought from a local supermarket called Giant Express Arief Rahman Hakim, Surabaya. The extraction method used was water-based maceration. The process of the extraction was done at the laboratory of the Pharmacology Department of Medical Faculty, Universitas Airlangga Surabaya. The ether, used for anesthesia for blood sample aspiration from the rats, was acquired from the Pharmacology Department of Medical Faculty, Universitas Airlangga Surabaya.

The samples were fifteen ( $\alpha=0.05$ ) male Wistar rats, as a reliable subject for triglyceride malabsorption (Merola et al 2011). The rats were all seven until eight weeks old, and they weren't on conditions that could disturb lipid absorption, such as on medications. Rats were fed the same diet, which are Pokphand and mineral water (Aqua) starting from a week prior to the first day of the test. To avoid any injuries due to possibilities of any contact between rats, partitions were applied in the cages. There was a washout period of 1 week between the two days of when the blood samples were taken. Prior to each day of the experiments, rats were fasted for sixteen hours prior to experiment to stabilize triglyceride levels at the time of the test, referring to a previous study (Merola et al 2011).

During the first day of the test, rats were administered 10 ml/kgBW of palm oil orally. During the second day of the test, rats were administered the 10 ml/kgBW of the same kind of palm oil orally, but with the addition of 33.3 mg/kgBW of *Glycine max* extract. On each time of the tests, blood samples were collected by cardiac puncture, after rats were anesthetized with ether, 120 minutes after the administration of palm oil with and without *Glycine max* extract, 120 minutes post-prandium being the time in which the highest level of post-prandial serum total triglyceride is achieved in Wistar rats, based on a previous study (Merola et al 2011).

The blood samples were then allowed to clot and then used to measure the total serum triglycerides. The measurements were done at the Clinical Pathology Department of RSUD Dr Soetomo in Surabaya with the help of its staff with a triglyceride assay kit (Siemens Dimension ® RxL Max ® Integrated Chemistry System, German) through biochromatic endpoint measurement.

The data of post-prandial serum triglyceride level compared is measured in miligrams per deciliter (mg/dL), which is a ratio data, in one group of sample. The method of data analysis used is the paired t test, if the distribution is normal. If not, Wilcoxon signed rank test could be used ( $\alpha=0.05$ ).

## RESULTS

This experiment was done to compare the 2-hour post-prandial serum triglyceride between rats administered with palm oil with and without edamame (*Glycine max*) extract through intragastric administration. Both the palm oil and edamame (*Glycine max*) extract are calculated in volume and mass, respectively, according to how much each rats weigh. Four subjects were excluded because of their deaths during the washout period. Each rat's weight and how much palm oil and edamame extract were given are presented in table 1.

The levels of 2-hour post-prandial serum triglyceride are shown in Table 2 below. The level of serum triglyceride in both treatments had normal distributions ( $p>0.05$ ). There was an increase in the mean level of 2-hour post-prandial serum triglyceride after the administration of edamame extract (69.3637 mg/dL). However, the difference wasn't statistically significant ( $p>0.05$ ).

Table 1. Rats weight, volume of palm oil administered, and mass of edamame (*Glycine max*) extract administered

Rats	Weight (grams)	Palm Oil Administered (10 cc/kgBW)	Edamame ( <i>Glycine max</i> ) Extract Administered (33.3 mg/kgBW)
1	138	1.38	4.595
2	150	1.5	4.995
3	153	1.53	5.095
4	145	1.45	4.829
5	154	1.54	5.128
6	133	1.33	4.429
7	145	1.45	4.829
8	142	1.42	4.729
9	144	1.44	4.795
10	170	1.7	5.661
11	127	1.27	4.229

## DISCUSSION

Although the difference of the mean 2-hour post prandial serum triglyceride between intragastric administration of palm oil with and without edamame (*Glycine max*) extract appears significantly different in a glance (69.37 mg/dL), the statistical analysis proves it to be not significantly different. This means that the first hypothesis that the researcher proposed, which was that edamame (*Glycine max*) extract administration will result in a lower post prandial serum triglyceride, was not in conformity with the result of this experiment. Even more, the mean serum triglyceride after edamame (*Glycine max*) extract administration was higher compared to when it wasn't administered, albeit the insignificance according to the statistical analysis.

Table 2. Serum triglyceride of rats after administration of palm oil without edamame (*Glycine max*) extract and palm oil with edamame (*Glycine max*) extract

Rats	Serum Triglyceride (mg/dL)	
	2 Hours After Palm Oil Administration	2 Hours After Palm Oil & Edamame ( <i>Glycine max</i> ) Extract Administration
1	100	34
2	79	497
3	35	70
4	89	198
5	244	408
6	142	67
7	101	118
8	112	127
9	93	95
10	445	319
11	135	405
Mean±SD	143.1818±112.792	212.5455±164.667

This nonconformity could possibly mean certain things. If the experiment was done correctly, it could mean that edamame (*Glycine max*) does not lower post-prandial serum triglyceride through lipid absorption inhibition through a way the researcher proposed. However, seeing a big discrepancy with the results of a previous study with a similar topic (Merola et al 2011), it would also be wise to consider the possibility of the process of the experiment being done not entirely free from errors, possibly through technical errors, such as ways of preparing subjects, sample taking, handling, and caring, sample assessment, etc. The previous study mentioned shows a rather insignificant variety compared to the results in this research. The previous experiment mentioned shows a 2-hour post-prandial triglyceride which are all less than 150 mg/dL, while in this research many exceeds that level.

It was proven in a previous study that the content of calcium from dairy products ingested was inversely related to the post-prandial chylomicron triglyceride, while calcium from a supplement wasn't (Lorenzen et al 2007). It was mentioned that the possible mechanism of how it happened, after ruling out other possible factors, was through inhibition of lipid absorption, such as the one proposed in this research. The study suggested that there could be another component alongside the calcium in the dairy product that helped inhibit the lipid absorption, which in that study was phosphate, while in the supplement it was calcium carbonate. It may be possible that the calcium in edamame (*Glycine max*) is different from the ones found in dairy products, which explains the result of this experiment. It could also be due to the quantity of how much edamame (*Glycine max*) extract is given. Incorrect dose could lead to the failure for a significant lipid absorption inhibition to

occur or the triglyceride content of the edamame itself masked the lipid absorption inhibition effect.

To assess the problems, an explanation of each major step and the possible technical errors that could occur during each is necessary. The selection of the subjects itself could disturb the results, if not done correctly. Although in this research the rats are selected through an inclusion and exclusion criteria, other unrecognized factors could come into play. Those factors could include diseases, in this case that could disturb lipid metabolism in the body. In this research, however, the rats are made sure that they have not been recently exposed to factors that could disturb lipid metabolisms, such as medications, although the diseases are not thoroughly assessed. Their diets are also homogenized in a certain period in the hopes of overcoming the chance of this from occurring, and moreover only male rats are selected for this experiment. Calculating the diets, which wasn't done in this experiment, prior to the experiments to an even more homogenized nutritional value would also be wise.

Lack of daily observations of test subjects could also be a contributing factor. There could be certain things that could happen to the rats that would disturb the experiment. This was prevented through partition application in the cages, since interactions between rats would be different for each rat if partitions were not applied.

Sample preparation and taking in this experiment could also be a major influence on the results. Extraction of samples could also influence the nutritional value in a given amount of volume, especially the active substances that are hypothesized to play a major role in an experiment, which in this study was dietary fiber and calcium. However, it was stated that water-based extraction using maceration technique on pequi (*Caryocar brasiliense* Camb.) peel significantly increases the total, soluble, and insoluble dietary fiber (Siqueira et al 2013). Although it wasn't soybean or edamame, the study could support the idea that it is possible water-based extraction using the maceration technique could result in an increased amount of dietary fiber in a given amount of volume. In this research, palm oil was used instead of lechitin oil, which was proven to show the most representative result for researches regarding oral lipid loading test for triglyceride malabsorption tests (Merola et al 2011). It was stated that it could be argued that lechitin oil probably produces better emulsion as well as smaller lipid particles which allow a better access of lipase, compared to others, although in that study palm oil was not included. Substitution of the control substances could also influence results. To overcome this, in the

future prologue experiment could be done to know the pattern of serum triglyceride in rats after with palm oil administration. In a study comparing post-prandial plasma triglyceride between palm oil and sunflower seed oil, it was concluded that although the total plasma triglyceride in a given amount of time was higher in rats fed with palm oil, relative hypertriglyceridemia found in palm oil-fed rats is due to less efficient catabolism, which in this case was the activity of lipoprotein lipase, and not to increased synthesis of plasma triglyceride (Groot et al 1988). This could support the idea that analyzing chylomicron triglyceride, instead of total serum triglyceride, would be better, which will be discussed later on. The total time needed to administer the substances to all rats were 15 minutes, similar to the time needed to withdraw blood from all of the rats. This would help in minimizing the occurrence for errors in the process. Application of anesthesia was intended to ease the blood aspiration process and, to a lesser extent, to prevent serum lipid markers alteration from stress if blood is withdrawn from the rat without anesthesia (Merola et al 2011). However, a study mentioned that anesthesia could alter biochemical serum markers, including serum triglyceride (Gil et al 2010). However, all rats are treated the same, which was through anesthesia with ether, which could be a way to prevent any confounding factors that could come into play in disturbing the research result. Cardiac puncture is a rather invasive way to withdraw blood. The decreased blood volume could also be the cause of death of the rats in this experiment, although these deaths don't affect the other rats still living after cardiac puncture. In regards to how much the blood is withdrawn, since the maximum amount of blood that should be withdrawn from a rat separated by a period of weeks is 1% in milliliters of rats body weight in grams (i.e., 1.5 ml from a 150 grams rat, 40 ml from a 4 kg rat), the amount of how much blood is withdrawn in this experiment would likely not disturb the result since every rats had their blood withdrawn for 1 ml and all of the rats weigh more than 100 grams (127 grams being the lowest and 170 grams being the high-est), and there is a washout period of one week for the content of the blood to return to normal. The process of blood aspiration itself could also allow certain factors to disturb the results. To prevent this, the process was done by a trained laboratory worker carefully to prevent the heart from collapsing, although it does not completely prevent other factors to come into play.

Sample caring, handling, and assessment could also influence the results. In this research, the blood samples were brought directly to the Clinical Pathology Department of RSUD Dr. Soetomo right after all samples are successfully withdrawn, which would prevent any factors that could influence the result from sending the samples to be assessed to long. The

assessment was done using a triglyceride assay kit (Siemens Dimension® RxL Max® Integrated Chemistry System, German) with the type of measurement being the biochromatic endpoint.

It should be taken into consideration that inadequate lipid absorption, resulting in accumulation of lipid in the lumen of the intestines, could lead to steatorrhea, which is the presence of fat in stools. Steatorrhea itself could result in a fatty diarrhea (Hanrahan & Rita 2016). However, a study showed that high fiber diet, although on patients with pancreatic insufficiency, increases fecal weight and fecal fat excretion, abdominal flatulence, but no increase in frequency of bowel movements (Dutta & Hlasko 1985). This is possibly due to the bulking effect dietary fiber possesses to stools in the intestine, making bowel movements soft and firm (University of Michigan Health System 2011).

## CONCLUSION

From this research, it could be concluded that edamame (*Glycine max*) extract had no effect on 2-hour postprandial serum triglyceride after palm oil administration via intragastric tube. However, a more thorough evaluation could be done to perfect this experiment, seeing the comparison between the results of this research to a previous study with a similar method and parameters, as discussed before (Merola et al 2011).

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