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Effects of Genetically Modified (GM) Soybean and Tempe Consumption on Blood Profile, Malondialdehyde (MDA) Level and Superoxide Dismutase (SOD) Activity of *Sprague-Dawley* Rats

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Abstract

Tempe, an Indonesian traditional food, is the product of the fermentation of soybean by the mold *Rhizopus oligosporus*. Tempe and other soyfoods products such as tofu, soy sauce and soymilk has long been a part of traditional Indonesian diets, consumed at daily basis and become important protein sources for most population. On the other side, soybean consumption requirements are fulfilled with imported soybeans which mostly are cultivated by genetic modification (Genetically Modified Foods or GM Foods). The use of GM soybean in the production of tempe raised many concerns, particularly on health and safety aspects. Utilization of GM soybean to produce tempe resulted in many different perspectives especially safety and health aspects.

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The objectives of this study were to evaluate effects of GM soybean and tempe consumption on liver and kidney malondialdehyde (MDA) activity, liver and kidney superoxide dismutase (SOD) activity and blood profile of *Sprague-Dawley* rat. There were 45 rats divided into eight interventions and one control groups, given tempe and soy ration either GM or conventional with concentration 10% and 20% for 90 days. Results showed that group which was given ration of 10% protein from conventional soybean had lower liver and kidney MDA levels if compared to GM tempe 10% and 20% groups, but was not significant compared to conventional soybean 20% and casein 10% groups. Meanwhile, liver and kidney SOD levels were not significant ($p>0.05$) between intervention groups. There were no significant differences on blood profile during intervention. Results of experimental rats MDA, SOD and blood profile analysis showed that GM soybean and tempe are safe for consumption, as safe as conventional soybean and tempe.

Keyword: GM Soybean; Tempe; MDA; SOD; *Sprague-Dawley* rats

1. Introduction

Soybean requirements for consumption of Indonesian people were abundant in 2013, national soybean requirements reached 2.2 million metric tons. About 98.4% of the national soybean requirements are used as raw material to produce tempe, tofu, soy sauce, soymilk etc. Approximately 1.2 million metric tons are used to produce tempe, 650 thousand tons to produce tofu and soy sauce and the rest to produce other foods. Data from Ministry of Agriculture in 1978-2008, growth rate of soybean consumption reached 7.22 % per year [1]. The quantity of national soybean consumption is inseparable from the benefit of the commodity. Protein quantity and quality of soybean are really high compared to other beans. Protein contained in soybean is 46.2 gram per 100 gram dry basis [2]. Besides its high protein content, soybean is also rich in vitamin and mineral. Previous research showed that soybean consumption could lower plasma cholesterol, triacylglycerol and blood glucose, also contains antioxidant [3,4,5].

Tempe and soyfood products have been widely consumed by Indonesian; they provide plentiful amounts of high-quality protein and many researches suggests soyfoods offer health benefits independent of the nutrients they provide [6]. As a source of protein for most Indonesian, tempe is consumed in greater quantities than other protein sources. Tempe supplies at least 10% of the current protein consumption, while chicken egg supplies 1.25%, meat supplies 3.15% and cereals supply around 60% [7].

Due to limited production of local soybean, national soybean requirements for the past five years (2010-2014), which were 2.3 million tons of dry beans per year, mostly were fulfilled by imported soybean from country that adopted transgenic soybean cultivation. Therefore, most soybeans used as raw material for tempe were Genetically Modified Organism (GMO). GM soybean is variety of soybean that has been genetically modified to produce soybean with various improvements, such as more resistant to diseases and pests, more resistant to herbicides and have bigger bean size.

On the other hand, there is negative perception towards transgenic foods because of fears on the new characteristics retained by the plants can express new protein as a result of other species' genes that may develop

toxicity or new allergy. This happens because of limited information on GMO distributed in the community based on responsible scientific facts. No scientific evidences about effects of GM soybean and tempe consumption on blood serum, hematology, MDA and SOD levels of liver and kidney have induced this research to be conducted.

This research was aimed to evaluate correspondence of nutrient quality of tempe made from GM and conventional soybean and the consumption effect on health profile of experimental rats, which are serum profile and hematology, also MDA and SOD of experimental rats' organs (liver and kidney).

2. Method

2.1 Raw Materials

Raw materials used to make tempe were GM and non-GM soybean (*Glycine max*) imported from USA that can be used to make tofu and tempe that were obtained from KOPTI of Bogor District. Non-GM soybean was packed in a special 30 kg package, accompanied by non-GM certificate from the supplier.

2.1.1 Experimental Rats

Experimental rats used were weaned male white rats of *Sprague dawley* strain that were obtained from National Agency of Drug and Food Control (NADFC) Jakarta, Indonesia.

2.2 Steps of Producing Tempe

Raw materials used in tempe production were GM and non-GM soybean (*Glycine max*). Tempe production process was conducted by applying Good Hygienic Practices (GHP) in Indonesian Tempe House (ITH) Bogor that has been certified with HACCP. The process were: cleaning and sorting the soybean, soaking with water for 1 hour, boiling for 30 minutes, re-soaking for 12 hours and peeling of the skin coat. Soybean that has been peeled off its skin coat was cleaned and separated from its grown sprout, and doused with hot water. After that, soybean was chilled and inoculated by *Rhizopus sp* evenly then packed and incubated for 40 hours at at 28^{o}C.

2.3 Ration Production Process

Ration productions for the experimental rats were differentiated by their protein sources, which were GM tempe ration, non-GM tempe ration, GM soybean ration, non-GM soybean ration and casein ration as the standard. Boiled tempe and soybean were made into powder to enable in mixing the raw materials of the ration. Ration was given based on daily requirements of the rats and arranged based on AOAC (2005).

2.4 Analysis of GM and Non-GM Soybean and Tempe Consumption Effects In Vivo

This study was approved by the Animal Ethic Committee of the Bogor Agricultural University. Analysis of GM and non-GM tempe powder effect in vivo used weaned male white rats of *Sprague dawley* that have been adapted for three days by giving casein ration (standard) and drinking water *ad libitum*. After adaptation period,

45 rats were selected based on similarity of body weight and were divided into nine groups, which were groups of rats fed with: (1) 10% protein from GM tempe, (2) 10% protein from non-GM tempe, (3) 20% protein from GM tempe, (4) 20% protein from non-GM tempe, (5) 10% protein from GM soybean, (6) 10% protein from non-GM soybean, (7) 20% protein from GM soybean, (8) 20% protein from non-GM soybean, and (9) 10% protein from casein. Each groups of rats had weight differences less than 10 grams and differences between rats within each groups maximal were 5 grams. Intervention was conducted for 90 days. During intervention period, observation on ration consumption was conducted every day and on body weight of rats every six days.

2.5 Preparation and Surgery of Organ Samples to be Analyzed

Surgery was conducted on experimental rats at the beginning of the study to obtain baseline data on rats' condition and 90 days after provision of the experimental ration. At the end of intervention, surgery was conducted on experimental rats, that have been fasting for one night. Rats were anesthetized using mix solution of ketamine and xylazine. Rats' organs (kidney, testis and liver) were taken by surgical scissors and tweezers. Then organs were weighted with analytical scale. Liver, kidney and testis tissue of the experimental rats were sampled to be analyzed histopathological to know the general morphology of those organs tissue.

2.6 Analysis of Malondialdehyde (MDA) Level [8]

Oxidative stress level was analyzed by measuring malondialdehyde (MDA) as a result of unsaturated fatty acid oxidation in the liver/kidney compared with TEP (tetraethoxypropane) standard curve. A 1 gram liver or kidney sample was smashed and homogenized by adding 4 mL of PBS (*phospate buffer saline*) solution 0.15 M. Homogenate then was centrifuged 3000 rpm with centrifugal radius 17.90 cm for 20 minutes until supernatant was clear. For analysis, 1 mL of liver supernatant or standard TEP working solution was mixed with 4 mL of cold HCl 0.25 N that contained TCA, TBA and BHT. Solution then was vortexed and heated 80^{o}C using water bath for 1 hour. After cooled, solution was centrifuged 3000 rpm. Then absorbance of clear supernatant was measured at lambda 532 nm and compared to TEP standard curve to calculate MDA level of sample.

2.7 Analysis of Superoxide Dismutase (SOD) Activity [8]

Liver or kidney sample was smashed and extracted by phosphate buffer pH 7, with ratio 1:10. Extraction sample was centrifuged with 3000 rpm speed and centrifugal radius 17.90 cm for 10 minutes in cold condition. Absorbance measurement was conducted by adding 2800 µl buffer sodium carbonate pH 10,2, 100 µl sample which was supernatant containing SOD and 100 µl epinephrine solution to the test tube. Then absorbance was read at lambda 480 nm at minute 1, 2, 3, and 4.

2.8 Statistical Analysis

Data processing was conducted using Microsoft Excel 2010 and SPSS 17.0 for Windows. Analysis was conducted by One Way ANOVA. If there was any significant difference, further test using Duncan test was conducted.

3. Result and Discussion

3.1 Haematological Analysis

Haematological analysis is used to measure the number of erythrocyte, hemoglobin, leucocyte, thrombocyte and hematocrit. Haematology is a sensitive indicator that describes rats' health generally [9]. The purpose of haematological analysis is to observe any hematological disorders such as number and function of blood cells, help in diagnosing infectious disease and identify systemic disorders of kidney and liver [10].

Table 1: Haematology profile of rats after 90 day intervention

Intervention Group	Haemoglobin (g/dL)	Leukocyte ($x10^3/mm^3$)	Thrombocyte ($x10^3/mm^3$)	Haematocrit (%)	Erythrocyte ($x10^6/mm^3$)
GM tempe 10%	13.34 ± 0.41^{ab}	8.58 ± 1.76^{bc}	611.40 ± 41.37^{a}	34.32 ± 1.12^{a}	7.65 ± 0.68^{abc}
GM tempe 20%	14.02 ± 1.17^{b}	6.92 ± 1.30^{abc}	633.40 ± 40.59^{a}	36.22 ± 2.70^{bc}	8.14 ± 0.93^{c}
N-GM tempe 10%	13.14 ± 0.45^{a}	5.50 ± 1.35^{a}	557.00 ± 41.66^{a}	33.84 ± 1.99^{ab}	7.73 ± 0.21^{abc}
N-GM tempe 20%	13.64 ± 0.94^{ab}	9.26 ± 3.25^{c}	634.60 ± 53.39^{a}	36.32 ± 1.77^{bc}	7.80 ± 0.33^{bc}
GM soybean 10%	13.24 ± 0.42^{ab}	5.90 ± 2.78^{a}	631.00 ± 33.21^{a}	35.32 ± 1.48^{abc}	7.59 ± 0.21^{ab}
GM soybean 20%	13.66 ± 0.29^{ab}	6.50 ± 1.75^{ab}	651.00 ± 53.18^{a}	36.76 ± 1.05^{c}	7.62 ± 0.29^{abc}
N-GM soybean 10%	13.68 ± 0.45^{ab}	6.90 ± 1.32^{abc}	634.60 ± 39.96^{a}	36.44 ± 1.82^{bc}	7.91 ± 0.23^{c}
N-GM soybean 20%	13.30 ± 0.73^{ab}	7.62 ± 1.62^{abc}	670.20 ± 91.34^{a}	35.26 ± 2.22^{abc}	7.41 ± 0.27^{a}
CASEIN	13.84 ± 0.72^{ab}	7.36 ± 2.31^{abc}	614.60 ± 62.47^{a}	36.56 ± 1.37^{c}	7.91 ± 0.44^{bc}

Remark: Values followed with the different letters in the same column showed significant difference ($p<0.01$) by Duncan test.

Haemoglobin (Hb) is an erythrocyte pigment consisting of conjugated protein complex that contained iron. The Hb protein is globin, and red colour is caused by heme colour. Heme is a compound containing one iron atom [11]. Analysis of variance (ANOVA) showed that intervention type had significant effect ($p<0.01$) on hemoglobin value. Further test with Duncan test showed that hemoglobin value of GM tempe 20% group was significantly higher than conventional tempe 10% group, but was not significantly different from GM tempe 10%, conventional tempe 20%, GM soybean 10% and 20%, conventional soybean 10% and 20% groups, also casein as control. This indicated that rats group fed by GM and conventional tempe and soybean ration from plant food can provide iron intake as good as casein from animal food. The iron level in soybean is 11 mg/100 gram and tempe is 9 mg/100 gram [2]. Hemoglobin levels of each experimental rat groups were normal. Normal hemoglobin value of experimental rat is 12-17.5 g/dl [12].

Leucocyte roles in the body are to defend organisms cellular and humoral against foreign substances. Leucocyte can be amoeboid and by diapedesis, leucocyte can leave blood capillary through the endothelial cells and penetrate the connecting tissue (Effendi 2003). Analysis of variance (ANOVA) showed that intervention type

had significant effect ($p<0.01$) on leucocyte value. Further test with Duncan test showed that leucocyte value of conventional tempe 20% was significantly higher than conventional tempe 10%, GM soybean 10% and GM soybean 20% groups, but was not significantly different from GM tempe 10% and 20%, conventional soybean 10% and 20% groups and also casein as control. Normal leucocyte value of experimental rat is 5-25x10^3/mm^3 [12].

Thrombocyte has an important role in blood clotting. In normal condition, thrombocyte circulates the body through blood flow. Low thrombocyte can cause prolonged time in blood clotting process. Analysis of variance (ANOVA) showed that type of intervention had no significant effect ($p>0.01$) on thrombocyte value (Table 1). Normal thrombocyte value of experimental rat is 140-450x10^3/mm^3 [14]. Numbers of thrombocyte in each experimental rat groups were more than normal. This was caused by several factors such as rat activity and body metabolism. Thrombocyte numbers that were more than normal caused by soybean and tempe powder consumption indicates that they can be used as alternative food to increase thrombocyte value which is low in dengue hemorrhagic fever patient [12].

Hematocrit can be used to diagnose normal condition, anemia and polycythemia. Hematocrit value can be influenced by psychological and pathological factors. Low hematocrit value indicates anemia or hemorrhage. High hematocrit value can be caused by dehydration of the specimen [16]. Analysis of variance (ANOVA) showed that intervention type had significant effect ($p<0.01$) on hematocrit value. Further test with Duncan test showed that hematocrit value of GM soybean 20% and casein groups were significantly higher than GM tempe 10% and conventional tempe 10% groups, but were not significantly different from GM tempe 20%, conventional tempe 20%, GM soybean 10%, conventional soybean 10% and 20% groups. Protein contents in ration consumed had effect on hematocrit level of rats. Low protein consumption can cause disorder in synthesis of erythropoietin hormone which controls the speed of red blood cells production in the bone marrow and stimulate cellular division process faster [17]. All groups of experimental rats had normal hematocrit level which is 33-50% [18].

Erythrocyte is red blood cell that has role carrying hemoglobin in the circulation. Erythrocyte is produced in bone marrow and spleen [11]. The main function of erythrocyte is to transport haemoglobin and thus carry oxygen from lungs to tissue [19]. Several important nutrients that are needed to produce erythrocyte are protein (amino acids), vitamins (vitamins B2, B6, B12, folate, thiamin, vitamin C and E) and minerals (Fe, Cu, Mn and Co). If body is deficient of one of those important nutrients, production of erythrocyte will be disrupted and it can cause anemia [20]. Further test with Duncan test showed that hematocrit value of GM tempe 20% group was significantly higher than GM soybean 10% and conventional soybean 20% groups, but was not significantly different from GM tempe 10%, conventional tempe 10% and 20%, GM soybean 20% groups and also casein as control. From the analysis of erythrocyte value, it was showed that all interventions gave adequate iron intake so that numbers of erythrocyte were normal. Normal erythrocyte value of experimental rat is 7.2 - 9.6 million/mm^3 blood [12].

Analysis of variance (ANOVA) showed that intervention type had significant effect ($p<0.01$) on cholesterol value. Further test with Duncan test showed that cholesterol value of GM tempe 10% group was significantly

higher than GM tempe 20%, conventional tempe 10% and 20%, GM soybean 10% and 20% and conventional soybean 10% and 20% groups, but was not significantly different than casein as control. Cholesterol values of all experimental rat groups were in the normal range which is 40-130 mg/dl [21]. This study proved that tempe and soybean consumption does not increase cholesterol level. This result was similar to earlier study that stated boiled tempe and soybean are foods that do not increase cholesterol level [22]. Previous researches reported that soybean can decrease total cholesterol level, triacylglycerol and blood glucose levels and have a role as potential antioxidant [3, 4, 23, 24].

Table 2: Blood serum profile of rats after 90-days intervention

Intervention Group	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
GM tempe 10%	68.14±16.26^{c}	47.14±4.81^{ab}	53.60±6.81^{b}	32.20±7.79^{ab}
GM tempe 20%	47.14±3.39^{ab}	68.43±16.03^{b}	51.27±9.02^{b}	26.20±2.51^{a}
N-GM tempe 10%	53.00±12.42^{ab}	41.57±12.71^{a}	45.57±9.68^{ab}	37.02±16.28^{ab}
N-GM tempe 20%	48.14±7.15^{ab}	52.00±14.96^{ab}	46.29±6.05^{ab}	39.60±14.88^{ab}
GM soybean 10%	52.86±6.57^{ab}	44.71±20.81^{a}	48.86±9.04^{ab}	38.40±9.83^{ab}
GM soybean 20%	42.57±6.78^{a}	46.86±14.61^{a}	37.29±8.20^{a}	43.60±17.02^{b}
N-GM soybean 10%	53.00±8.83^{ab}	42.14±13.48^{a}	46.57±6.08^{ab}	31.20±5.86^{ab}
N-GM soybean 20%	42.71±9.29^{a}	46.14±13.42^{a}	46.14±5.30^{ab}	28.40±6.68^{ab}
CASEIN	59.57±12.43^{bc}	55.80±15.96^{ab}	54.43±11.63^{b}	32.40±5.47^{ab}

Remark: Values followed with the different letters in the same column showed significant difference ($p<0.01$) by Duncan test.

Analysis of variance (ANOVA) showed that intervention type had significant effect ($p<0.01$) on triglyceride value. Further test with Duncan test showed that triglyceride value of GM tempe 20% group was significantly higher than conventional tempe 10%, GM and conventional soybean 10 % and 20% groups, but was not significantly different from GM tempe 10%, conventional tempe 20% and casein as control. Triglyceride values of all experimental rat groups were in the normal range which is 26-145 mg/dl [25]. This study showed that tempe and soybean consumption does not increase triglyceride value. The triglyceride value as result of this study was very influenced by ration consumed. The increase and decrease of blood triglyceride level is influenced by total fat consumed.

Analysis of variance (ANOVA) showed that intervention type had significant effect ($p<0.01$) on HDL value (Table 2). Further test with Duncan test showed that HDL value of GM tempe 10%, GM tempe 20% and casein groups were significantly higher than GM soybean 20%, but was not significantly different from conventional tempe 10% and 20%, GM soybean 10%, conventional soybean 10% and 20% groups. HDL values of all experimental rat groups were higher than normal which is >35 mg/dl [26]. This study proved that tempe and soybean consumption can increase HDL level. This result was similar to previous study which stated that tempe can be an alternative food to increase blood HDL, therefore decrease incidence of fat accumulation in the

vascular system [22].

Analysis of variance (ANOVA) showed that intervention type had significant effect ($p<0.01$) on LDL value (Table 2). Further test with Duncan test showed that LDL value of GM soybean 20% group was significantly higher than GM tempe 20% group, but was not significantly different from other tempe and soybean groups, and also casein as control. LDL values of all experimental rat groups were in the normal range which is <100 mg/dl [27]. This study proved that tempe and soybean consumption does not increase LDL value. It is in line with previous study that stated black soybean supplementation to rat can decrease LDL cholesterol level by 60%. Isoflavon in soybean has effect on LDL receptors. This is like estrogen which also has effect to increase up regulating activity of LDL receptors. The increasement of LDL receptors will increase LDL clearance from blood circulation so that numbers of LDL cholesterol in blood will decrease [28].

Table 3: Blood serum profile of rats after 90-days intervention

Intervention Group	Ureum (mg/dl)	Creatinin (mg/dl)	Protein (g/dl)	Albumin (g/dl)
GM tempe 10%	25.10 ± 5.17^{a}	0.74 ± 0.10^{ab}	5.82 ± 0.55^{ab}	3.11 ± 0.48^{ab}
GM tempe 20%	33.63 ± 4.45^{b}	0.64 ± 0.07^{a}	6.28 ± 0.48^{abc}	3.12 ± 0.24^{ab}
N-GM tempe 10%	25.53 ± 3.74^{ab}	0.71 ± 0.14^{ab}	5.64 ± 0.55^{a}	2.93 ± 0.18^{a}
N-GM tempe 20%	37.08 ± 9.11^{b}	0.67 ± 0.08^{ab}	6.41 ± 0.52^{bc}	3.33 ± 0.20^{b}
GM soybean 10%	23.34 ± 4.75^{a}	0.69 ± 0.11^{ab}	6.48 ± 0.79^{bc}	3.08 ± 0.22^{ab}
GM soybean 20%	34.06 ± 5.08^{b}	0.71 ± 0.14^{ab}	6.45 ± 0.21^{bc}	3.13 ± 0.17^{ab}
N-GM soybean 10%	27.16 ± 7.11^{ab}	0.72 ± 0.14^{ab}	5.86 ± 0.50^{ab}	3.15 ± 0.09^{ab}
N-GM soybean 20%	36.98 ± 5.07^{b}	0.66 ± 0.09^{a}	6.38 ± 0.85^{bc}	3.34 ± 0.25^{b}
CASEIN	32.47 ± 6.31^{b}	0.84 ± 0.16^{b}	6.59 ± 0.52^{c}	3.36 ± 0.33^{b}

Remark: Values followed with the different letters in the same column showed significant difference ($p<0.01$) by Duncan test.

High ureum level in blood indicates there is disorder in liver and kidney function. Liver and kidney disorder can be seen from liver and kidney condition such as enlargement. Analysis of variance (ANOVA) showed that intervention type had significant effect ($p<0.01$) on ureum value. Further test with Duncan test showed that ureum value of GM tempe 20%, conventional tempe 20%, GM soybean 20%, conventional soybean 20% and casein groups were significantly higher than GM tempe 10%, conventional tempe 10%, GM soybean 10% and conventional soybean 10% groups. Ureum values of all experimental rat groups were higher than normal range which is 2.67-3.44 mg/dl [29]. High ureum level in each groups of experimental rat might be due to the quantity of protein consumed. Ureum is a metabolic waste of protein. Therefore, high ureum level is affected by the quantity of protein consumed [30].

Statistical analysis (ANOVA) indicates that types of treatment gave a very significant effect ($p<0.01$) on creatinine level (Table 3). Further test with Duncan test showed that creatinin value of casein group was

significantly higher than GM tempe 20% and conventional soybean 20% groups, but was not significantly difference from GM and conventional tempe 10%, conventional tempe 20%, GM soybean 10% and 20% and conventional soybean 10% groups. Creatinin values of all experimental rat groups were higher than normal range which is 60.39-75.97 mg/dl [29]. High creatinin level in each groups of experimental rat might be due to the quantity of protein consumed. Creatinin is a metabolic waste of muscular protein. Therefore, high creatinin level is affected by the quantity of protein consumed.

Analysis of variance (ANOVA) showed that intervention type had significant effect ($p<0.01$) on protein value. (Table 3). Further test with Duncan test showed that protein value of casein group was significantly higher than GM and conventional tempe 10% and conventional soybean 10% groups, but was not significantly different from GM tempe 20%, conventional tempe 20%, GM soybean 10% and 20% and conventional soybean 20% groups.

Analysis of variance (ANOVA) showed that intervention type had significant effect ($p<0.01$) on albumin value (Table 3). Further test with Duncan test showed that albumin value of conventional tempe 20%, conventional soybean 20%, and casein groups were significantly higher than conventional tempe 10%, but was not significantly different from GM tempe 10% and 20%, GM soybean 10% and 20% and conventional soybean 10% groups. Albumin values of all experimental rat groups were in normal range which is 3-3.5 g/dl [31]. This result proved that consumption of tempe and soybean from GMO and conventional source can maintain blood albumin level. This result was similar to previous research which stated that plasma albumin levels of inpatients tend to be low. Tempe and soybean can be used as alternative foods for inpatients who tend to have low albumin level to increase their albumin level back to normal [32].

Table 4: Blood and electrolyte profiles of rats after 90-days intervention

Intervention Group	Random Blood Glucose (mg/dl)	Uric Acid (mg/dl)	AST (U/L)	ALT (U/L)
GM tempe 10%	210.80±44.32^{a}	0.58±0.22^{ab}	92.29±22.39^{bc}	44.43±6.65^{abc}
GM tempe 20%	225.40±44.13^{a}	0.58±0.31^{ab}	64.20±22.65^{a}	38.86±9.30^{abc}
N-GM tempe 10%	195.80±32.27^{a}	0.50±0.28^{ab}	79.43±8.66^{abc}	49.57±7.52^{c}
N-GM tempe 20%	230.20±53.23^{a}	0.64±0.20^{ab}	83.14±14.02^{abc}	47.00±13.76^{bc}
GM soybean 10%	237.00±41.10^{a}	0.70±0.16^{ab}	71.57±11.34^{ab}	34.86±4.49^{a}
GM soybean 20%	227.60±32.16^{a}	0.58±0.24^{ab}	98.71±21.01^{c}	45.71±7.39^{bc}
N-GM soybean 10%	223.40±35.94^{a}	0.84±0.27^{b}	89.57±16.96^{bc}	42.43±4.65^{abc}
N-GM soybean 20%	203.40±48.33^{a}	0.42±0.28^{a}	72.57±14.71^{ab}	36.80±5.66^{ab}
CASEIN	210.80±50.21^{a}	0.62±0.24^{ab}	90.43±22.25^{bc}	37.43±7.52^{ab}

Remark: Values followed with the different letters in the same column showed significant difference ($p<0.01$) by Duncan test.

Analysis of variance (ANOVA) showed that intervention type had no significant effect ($p>0.01$) on random blood glucose value (Table 4). Experimental rats that were given GM soybean 10% had the highest random blood glucose value which was 237 mg/dl. On the contrary, Experimental rats that were given conventional tempe 10% had the lowest random blood glucose value which was 195.80 mg/dl. Random blood glucose values of all experimental rats groups were higher than normal which is 105.2 mg/dl [32].

Analysis of variance (ANOVA) showed that intervention type had significant effect ($p<0.01$) on uric acid value (Table 4). Further test with Duncan test showed that uric acid value of conventional soybean 10% group was significantly higher than conventional soybean 20% group, but was not significantly different from GM and conventional tempe 10% and 20%, GM soybean 10% and 20% groups, and also casein as control. Uric acid levels of all experimental rat groups were in normal range which is 0.5-1.4 mg/dl [32]. This result could prove that tempe and soybean consumption from FMO and conventional source does not increase uric acid level. This result was similar to previous research which stated that consumption of boiled tempe and soybean powder does not have bad effect on blood uric acid level [22].

Analysis of variance (ANOVA) showed that intervention type had significant effect ($p<0.01$) on AST value (Table 4). Further test with Duncan test showed that AST value of GM soybean 20% group was significantly higher than GM tempe 20%, GM soybean 10% and conventional soybean 20% groups, but was not significantly different from GM tempe 10%, conventional tempe 10% and 20%, conventional tempe 10% groups and casein as control. AST levels of all experimental rat groups were lower than normal which is 141 U/L [34]. This result proved that consumption of tempe and soybean from GMO and conventional source can lower AST level in blood.

Analysis of variance (ANOVA) showed that intervention type had significant effect ($p<0.01$) on ALT value (Table 4). Further test with Duncan test showed that ALT value of conventional soybean 10% group was significantly higher than GM soybean 10%, conventional soybean 20%, and casein groups, but was not significantly different from GM tempe 10% and 20%, conventional tempe 20%, GM soybean 20% and conventional soybean 10% groups. AST levels of all experimental rat groups were lower than normal which is 12.6 U/L [34]. High AST level in blood is related to high activity of liver. This can be proved by measuring organ relative weight of each intervention groups in Table 5. It can be seen that liver relative weight of each intervention groups were not significantly different.

3.2 Ratio of Liver and Kidney Organs Weight to Body Weight

Growth of organ weight was directly proportional to growth of rat's body weight. Data in Table 5 shows that liver and kidney relative weight were not significantly different ($p>0.01$) in each intervention groups. This was caused by protein intake in all intervention groups were not different. Protein is composed of amino acids as building blocks of the body [35].

3.3 MDA Level in the Liver and Kidney

Malondialdehyde (MDA) is a result of polyunsaturated fat oxidation process by free radicals in the body;

therefore MDA can be used as an indicator of free radicals presence and indicator of oxidative damage to cell membranes in the body [36].

Table 5: Relative kidney dan liver weight of rats after 90-days intervention (g per 100 g body weight)

Intervention Group	Kidney Weight	Liver Weight
GM tempe 10%	0.25±0.0002[a]	2.49±0.002[a]
GM tempe 20%	0.27±0.0001[a]	2.55±0.002[a]
N-GM tempe 10%	0.24±0.0001[a]	2.44±0.001[a]
N-GM tempe 20%	0.26±0.0002[a]	2.49±0.002[a]
GM soybean 10%	0.27±0.0003[a]	2.61±0.002[a]
GM soybean 20%	0.26±0.0002[a]	2.63±0.002[a]
N-GM soybean 10%	0.25±0.0002[a]	2.56±0.001[a]
N-GM soybean 20%	0.26±0.0003[a]	2.63±0.002[a]
CASEIN	0.26±0.0003[a]	2.68±0.002[a]

Remark: Values followed with the same letters in the same column indicate no significant difference ($p>0.05$) by Duncan test.

Table 6: MDA & SOD level in the rats' liver and kidney after 90-days intervention

Intervention Group	Liver MDA (µmol/g)	Kidney MDA (µmol/g)	Liver SOD (unit/mg protein)	Kidney SOD (unit/mg protein)
GM tempe 10%	31.27±9.44[bc]	19.62±9.44[a]	412.79±61.21[a]	439.67±29.27[a]
GM tempe 20%	12.72±2.05[a]	10.76±2.05[a]	332.14±41.39[a]	412.79±53.76[a]
N-GM tempe 10%	30.05±4.39[bc]	17.57±4.39[a]	332.14±65.44[a]	430.71±34.32[a]
N-GM tempe 20%	21.00±3.49[ab]	17.43±3.49[a]	385.91±35.84[a]	430.71±17.92[a]
GM soybean 10%	15.67±1.62[a]	11.79±1.62[a]	359.03±53.76[a]	448.63±17.92[a]
GM soybean 20%	16.94±5.73[a]	9.14±5.73[a]	350.07±68.63[a]	448.63±17.92[a]
N-GM soybean 10%	40.07±7.11[c]	14.93±7.11[a]	376.95±73.89[a]	403.83±58.53[a]
N-GM soybean 20%	21.54±3.71[ab]	15.85±3.71[a]	367.99±97.06[a]	457.59±20.69[a]
CASEIN	17.15±6.22	12.87±6.22	323.18±73.89	448.63±17.92

Remark: Values followed with the different letters in the same column showed significant difference ($p<0.01$) by Duncan test.

Liver and kidney are important organs to know toxicity effect. Liver organ that used for MDA and SOD analysis is an organ which main function is nutrients storage, metabolism and biosynthesis. On the other hand, kidney is an organ which function is excrete metabolism wastes. Result analysis of MDA and SOD of rat's liver and kidney are shown in Table 6. Intervention type had significant effect ($p<0.01$) on liver MDA value, but had no significant effect on kidney MDA value of rat. Further test with Duncan test showed that liver MDA value of

GM soybean 20% group was significantly higher than non-GM tempe 10% and 20%, GM soybean 10%, non-GM soybean 10% and 20% groups, but was not significantly different from GM tempe 10% and 20% groups. Total isoflavon level in raw soybean is 140 mg per 100 gram and in tempe is 50 mg per 100 gram [2].

Antioxidant activity can neutralized free radicals in the body by preventing Reactive Oxygen Species (ROS) formation, this prevention involved superoxide dismutase (SOD) enzyme [38]. This enzyme has an important role in body defense system, especially towards reactive oxygen compound activity that causes oxidative stress. The highest SOD activity is found in the liver. Besides in the liver, SOD is also found in adrenal gland, kidney, blood, spleen, pancreas, brain, lungs, gut, intestine, ovaries and thymus [39]. Intervention types had no significant effect on SOD value in liver and kidney. Therefore it could be concluded that isoflavon content in GM and non-GM tempe and soybean can increase SOD level in liver and kidney of experimental rats to inhibit the formation of free radicals.

4. Conclusion

Assessment result of MDA, SOD and hematology showed that consumption of GM and non-GM tempe in long term does not cause any disorder or oxidative stress (free radicals) in experimental rats. This is supported by no various disorders was found in the rats during intervention period. Therefore GM soybean and tempe are safe to be consumed, they are as safe as non GM soybean and tempe.

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