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Consumption effect comparison of transgenic and non-transgenic soybean flour on antioxidant superoxide dismutase and spermatogenic cells profile of experimental rats

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Abstract

A 90-day subchronic toxicity study was conducted to compare the consumption effect of transgenic (imported) and non-transgenic (imported and local-Grobogan) soybean flour against the superoxide dismutase (SOD) and its effect on the spermatogenic cells profile of rats. Sprague Dawley rats were given diets formulated with either 10% or 20% protein of soybean flour and compared with 10% casein protein as a control group. At the end of the study, the internal organs (kidney and liver) were collected and analyzed for the malondialdehyde (MDA) and superdioxidase (SOD) levels. The testicular tissues were embedded in paraffin and stained using an immunohistochemical technique to obtain Cu, Zn-SOD levels. The findings indicated that the consumption of various soybean types and different concentrations did not significantly affect ($p>0.05$) on SOD levels of the kidney and liver, nor did it significantly affect ($p>0.05$) on MDA levels of the kidney, but it had a significant impact on liver MDA levels. MDA and SOD levels in the kidney and liver of all groups of rats did not, however, significantly deviate from those found in earlier research. The results also showed that the administration of transgenic and non-transgenic soybean flour provided the same benefits as casein on the Cu, Zn-SOD levels in the testicular tissues. Furthermore, local-Grobogan soybean flour significantly increased the number of spermatogenic cells and Cu, Zn-SOD levels compared to all treatments. The spermatozoa profile in all groups of rats also showed no significant difference ($p>0.05$) in the macroscopic profile (color, pH, and consistency) and microscopic profiles (mass and individual movements, motility, abnormalities, and concentration) of spermatozoa, indicating the absence of any adverse effect in the testicular tissues of experimental rats.

1. Introduction

Soybean is one of the most important commodities due to its high demand. According to the data from the Central Bureau of Statistics (Bandar Pusat Statistik (BPS), 2018a, 2018b), annual soybean production in Indonesia reaches 982.598 tons while productivity reaches 0.8 tons/Ha. The Ministry of Agriculture stated that the demand for soybeans reaches 2.67 tons annually to meet the direct household consumption, seeds, and industry demands. The high supply of imported soybeans compared with local soybeans affects the industry or tempe and tofu artisans to favour imported soybeans over the local varieties (Astawan *et al.*, 2013). Most of the imported commodities originate from countries that adopt the cultivation of genetically modified crops, which is the insertion of genetic material that has desired characteristics and is known as transgenic products. In transgenic soybeans, the commonly preferred characteristic is tolerance against glyphosate-active herbicides. The Indonesian FDA regulation No. 6 of 2018 permits the distribution of transgenic soybean which has been tested for its safety. However, cautionary remains a necessity, thus *in vivo* sub-chronic safety assessment on experimental animals needs to be done (Suwarno *et al.*, 2014).

Reactive oxygen species (ROS) is a free radical product from normal cell metabolism. The free radicals

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cause lipid peroxidation in the body due to the presence of unsaturated fatty acids, which results in unstable hyper-peroxides which easily convert into their aldehyde form (Yustika *et al.*, 2013). MDA is one of the secondary products of lipid peroxidation which has the mutagenic trait and is commonly used to measure the activity of free radicals in the body (Anggraeni *et al.*, 2017). SOD is an endogenic enzyme that acts as an antioxidant to protect cells from oxidative stress. The secondary structures of SOD, particularly Cu, Zn-SOD, Mn-SOD, and Fe-SOD are capable of capturing free reactive electrons, which can prevent further damage in cells (Astuti, 2008; Jadhav *et al.*, 1996). In addition to an endogenic antioxidant, an exogenic antioxidant is also capable of increasing cell protection, such as the isoflavones compound. Soy isoflavones consist of malonyl-glycosides, acetyl-glycosides, glycosides, and aglycones. Aglycone isoflavones have relatively high bioactivity compared with other types of isoflavones and consist of genistein, daidzein, and glycitein (Tipkanon *et al.*, 2010).

The liver organ functions are to detoxify and desaturate the fatty acids. If the liver microsome cell membrane is damaged by the presence of xenobiotics, the enzyme activities will be interrupted and cause toxicity (Yuslianti, 2018). The kidney filters the plasma and secretes it in urine, while the necessary nutrients are returned to the blood. The damage to the proximal tubule in the kidney will result in oxidative stress. The damage can be prevented by either antioxidant bioavailability in the body or exogenic antioxidants from food or supplement consumption (Zulfiani *et al.*, 2013).

The testicle is the main gland in the male reproductive system which secretes spermatozoa and androgens such as testosterone. The cell membranes of spermatogenic cells consist of a large number of polyunsaturated fatty acids. If PUFA interacts with free radicals, it will result in lipid peroxidation in the cells which increases membrane fluidity, membrane integrity disruption, and deactivation of membrane binding with enzymes and receptors (Sukmaningsih *et al.*, 2011). Normal spermatozoa can be characterized by its morphology and wholeness, progressive motility, and others (Feradis, 2010; Dwitarizki *et al*., 2015). Spermatozoa come from the spermatogenesis process in the seminiferous tubule, which starts from spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and finally spermatozoa.

Not much is known about the effects of transgenic and non-transgenic soybeans (imported and local) on the SOD antioxidant content in the testes in supporting the spermatogenesis process and its effect on the feminism issue. Therefore, this study aimed to determine the effects of transgenic and non-transgenic (imported and local) soybean consumption on the profiles of spermatozoa cells and spermatogenic, as well as SOD antioxidant content on the liver, kidney, and testicular tissue of experimental rats through *in vivo* sub-chronic test for 90 days.

2. Materials and methods

2.1 Materials

The main ingredients used in this study were local soybean Grobogan variety and imported transgenic US Origin (Event GTS 40-3-2) and non-transgenic which was obtained from KOPTI Bogor, as well as casein as standard ration. The materials which were used for analysis consisted of Bouin solution, graded alcohol (70, 80, 90, 95, and 100%), xylol solution, paraffin, phosphate buffer saline (PBS) solution, H_2O_2 solution, 10% normal serum, background sniper, Trekkie Universal Link, Avidin-HRP, diaminobenzidine (DAB), Cu, Zn-SOD antibody, and entellan®. The equipment included a syringe, petri dish, minor surgical tools, labels, glove, razor blade, bottles, beaker glass, measurement glass, filter paper, stirrer, tissue paper, aluminum foil, tissue basket, incubator, embedding tools, microtomes, object-glass, and microscope.

2.2 Soybean flour production

The soybean flour production process referred to Astawan *et al.* (2020), was sortation, washing 1, soaking, washing 2, and boiling at 100℃ for 30 mins, epidermis dehulling, draining, and drying on tray cabinet at 60℃ for 6 hrs, milling with disc mill, and 80-mesh sieving.

2.3 Soybean flour proximate analysis and experimental rats ration formulation

The proximate analysis referred to the Association of the Official Analytical Collaboration (AOAC) International method (2012), which consisted of water content (oven method), ash content (dry ashing method), fat content (Soxhlet method), crude protein content (Kjeldahl method), fiber content (strong acid-base method), and carbohydrate content which was calculated based on by difference method. The proximate analysis was used as a reference for ration formulation. The proteins used in this study were imported transgenic flour, non-transgenic flour, local Grobogan soybean flour, and casein as control.

2.4 Experimental rat treatments

The test subjects which was used in this study were white male Sprague-Dawley rats with weaning age of 21 days. The adaptation period was 7 days, and the rats were given a ration that consisted of 10% protein from

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casein. Then, 35 rats were chosen and divided into 7 treatment groups based on the protein source and content incorporated in the ration, namely: 10% casein (10% casein), 10% transgenic soybean flour (Trans SF 10%), 20% transgenic soybean flour (Trans SF 20%), 10% non-transgenic soybean flour (Ntrans SF 10%), 20% non-transgenic soybean flour (Ntrans SF 20%), 10% local Grobogan soybean flour (Grb SF 10%), and 20% local Grobogan soybean flour (Grb SF 20%).

At the beginning of treatment, the weight variation between rats within one group did not exceed 10 grams, and the variation between groups did not exceed 5 grams (Astawan *et al.* 1994). The provision of ration and drinking water was done *ad libitium*. Then, *in vivo* sub-chronic test was performed referring to the EFSA guideline (European Food Safety Authority (EFSA) Scientific Committee, 2011). Protein content from soybean flour in the ration was intentionally made 10 and 20% to evaluate the possible effects of the consumption of transgenic commodities.

2.5 Dissection of experimental rats and tissue processing

The dissection procedure for experimental rats was based on ethical clearance AUAC No. 06 obtained from IPB University. The rats were given anaesthesia which consisted of 70 mg/kg BW ketamine and xylazine 20 mg/kg BW (Wresdiyati *et al.*, 2018). The organs (liver, kidney, and testicles) were washed in physiological salt (NaCl 0.95%), and then weighed, for the testicular organ, was put into Bouin fixative solution before the process for tissue colouring. The testicular tissue processing was done by the standard embedding method using paraffin. Then, the tissue was sliced using a microtome and coloured using haematoxylin-eosin colouring (Kiernan, 1990) (Kiernan 1990), while for immunohistochemistry colouring, superoxide dismutase monoclonal antibody was used (Wresdiyati *et al.*, 2006, 2010).

2.6 Malondialdehyde and superdioxidase content analysis

The analysis of MDA content and SOD in the liver and kidney organs were performed by referring to Astuti *et al.* method (2009). MDA analysis was done using a spectrophotometer and compared with the tetraethoxypropane (TEP) standard curve. The SOD concentration was determined using spectrophotometry with a pure SOD standard curve (Astuti *et al.*, 2009; Maskar *et al*., 2015; Kusumaningrum *et al.*, 2017).

2.7 Profile of spermatogenic and spermatozoa analysis

The profile analysis of spermatogenic was done by counting the number of spermatogonium, primary spermatocytes, early spermatids, and late spermatids cells inside the testicular organ in the stage VIII wave of seminiferous tubule (Wing and Christensen, 1982). The spermatozoa profile analysis consisted of macroscopic and microscopic analysis. The macroscopic analysis includes color test and spermatozoa consistency which was done through direct observation. The microscopic spermatozoa analysis was done by analyzing the individual movement, mass movement, motility, abnormality, and concentration or the total number of spermatozoa cells. The spermatozoa concentration analysis was done based on Simbolon *et al.* (2013). The spermatozoa abnormality assessment referred to Maskar *et al.* (2015). The spermatozoa motility analysis was done by observing the semen motion, which consisted of individual motion, mass motion, backward motion, wavy, or rotating, and progressive movement speed (Garner and Hafez, 2002; Feradis, 2010).

2.8 Data analysis

The data analysis was carried out quantitatively and qualitatively. The quantitative analysis included proximate analysis, levels of MDA and SOD, spermatozoa microscopic profile, and spermatogenic cell profile. The analysis was done using a One-Way ANOVA test and proceeded with a post hoc Duncan test at a 95% confidence level to determine the effects of soybean type on the pre-determined parameters. The qualitative analysis was done by observing macroscopic spermatozoa profile, spermatogenic cell morphology, and Cu, Zn-SOD antioxidant levels in the testicular tissue.

The level of Cu, Zn-SOD in testicular tissue was visualized with brown color on the nucleus and cytoplasm. The observation was done based on the appearance of color intensity. The darker the brown color, the higher the Cu, Zn-SOD found in the cell. In the cell that does not contain Cu, Zn-SOD will appear blue (hematoxylin) in the nucleus due to the counterstain. The cell count was done at 10× magnification in random five fields of view in each tissue preparation following the method by Wresdiyati *et al.* (2006) by using the McMaster Biophotonics Image J program.

3. Results and discussion

3.1 Proximate analysis

The chemical analysis of casein and various types of soybean flour can be seen in Table 1. The analysis of variance results on water content, ash content, and fat content of the three types of soybean flour showed significantly different results ($p<0.05$), while protein, carbohydrate, and fiber content of the three types of soybean flour did not significantly different ($p>0.05$). However, the protein content of casein was significantly

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Table 1. Chemical composition of soy flour and casein.

Sample	Moisture (%wb)	Ash (%db)	Protein (%db)	Fat (%db)	Carbohydrate (%db)	Fibre (%db)
Trans SF	3.5±0.4[a]	3.6±0.0[b]	48.4±1.8[a]	31.1±0.3[d]	16.8±1.6[a]	7.8±0.0[b]
Ntrans SF	4.2±0.0[b]	3.7±0.0[c]	43.8±7.3[a]	29.8±0.1[c]	22.7±7.1[a]	7.8±0.0[b]
Grb SF	4.0±0.0[ab]	3.2±0.0[a]	49.7±1.1[a]	26.4±0.4[b]	20.7±0.6[a]	7.6±0.0[b]
Casein	4.2±0.2[b]	4.2±0.1[d]	79.2±52.9[b]	0.24±0.0[a]	16.4±0.4[a]	0.4±0.0[a]

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$). Trans SF: Transgenic soy flour, Ntrans SF: Non-transgenic soy flour, Grb SF: Local Grobogan soy flour.

higher than the three types of soybean flour ($p<0.05$). The proximate analysis was used as a reference for ration formulation.

The difference in moisture content, ash content, and fat content of the three types of soybean flour was caused due to the quality of post-harvest soybeans, such as handling, drying, distribution, and soybean storage processes (Astawan *et al.*, 2013). The higher the temperature and the longer the drying process, the lower the water content due to the evaporation of free water molecules from the material. The difference in ash content in soybean showed that the mineral content in each soybean flour was different. The mineral content in soybeans is commonly calcium, phosphor, potassium, and magnesium (Karr-Lilienthal *et al.*, 2004). The fat content was higher in transgenic soybeans compared with other samples, but the crude protein content in all three types of soybeans was not significantly different.

3.2 Profile of malondialdehyde and superdioxidase levels of kidney and liver

The MDA and SOD analyses in the kidney and liver are shown in Table 2. The types of soybean flour, protein concentration, and interaction between both treatments showed no significant differences ($p>0.05$) in the level of MDA in the kidney. The kidney MDA from experimental rat groups which were given 10% non-transgenic soybean flour and 10% Grobogan soybean flour were significantly lower ($p<0.05$) than in experimental groups which were given 10% casein. The lower the MDA level, the fewer free radicals are formed.

The level of free radicals can be suppressed with the aid of antioxidants. This was shown by the SOD level in the kidney which was not significantly different ($p>0.05$) in soybean flour treatment, protein concentration treatment, and interaction between both. The same goes with the group which was fed with soybean flour ration compared with the group that was given casein ration which showed no significant differences ($p>0.05$).

The type of soybean flour treatment, protein concentration treatment, and both interactions showed significant differences ($p<0.05$) in the level of liver MDA. The group which was given 10% Grobogan soybean flour had the lowest liver MDA level compared with another level of liver MDA in other treatment groups. Likewise, when the protein concentration was increased to 20%, the liver MDA in all groups was increased as well. However, experimental rats which were given Grobogan soybean flour resulted in the lowest level of liver MDA compared with imported transgenic and non-transgenic soybean flour treatments.

The analysis result of the level of liver SOD showed no significant differences ($p>0.05$) in types of soybean flour, protein concentration, and interaction between both. The level of liver MDA in 20% transgenic and non-transgenic soybean flour groups was significantly higher ($p<0.05$) than in the 10% casein group, while the SOD level of the group with various soybean flour treatments was not significantly different ($p>0.05$) compared with

Table 2. MDA and SOD profiles in the kidney and liver of experimental rats after 90 days of subchronic test.

Treatments	MDA Kidney (µmol/g)	SOD Kidney (U/mg protein)	MDA Liver (µmol/g)	SOD Liver (U/mg protein)
Trans SF 10%	11.8±1.9[a]	448.6±17.9[a]	15.7±1.6[b]	359.0±53.8[a]
Trans SF 20%	14.9±8.7[a]	403.8±58.5[a]	40.1±7.1[f*]	377.0±73.9[a]
Ntrans SF 10%	9.1±0.9[a*]	448.6±17.9[a]	16.9±5.7[c]	350.1±68.6[a]
Ntrans SF 20%	15.8±5.4[a]	457.6±20.7[a]	21.5±3.7[e*]	368.0±97.1[a]
Grb SF 10%	8.7±1.6[a*]	448.6±17.9[a]	10.2±1.3[a]	395.0±67.8[a]
Grb SF 20%	10.0±1.9[a]	448.6±17.9[a]	17.4±4.4[d]	350.1±62.1[a]
Casein 10%	12.9±1.4	448.6±17.9	17.1±6.2	323.2±73.9

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$) to casein, based on the T-test. Trans SF: Transgenic soy flour, Ntrans SF: Non-transgenic soy flour, Grb SF: Local Grobogan soy flour.

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10% casein ration treatment.

MDA and SOD levels in the kidney of experimental rats were not different from the previous study, with kidney MDA levels of 8.0 µmol/g and kidney SOD levels of 439.6 µmol/g (Astawan *et al.*, 2015). Meanwhile, in another study, kidney MDA levels ranged between 9-15 µmol/g while kidney SOD levels ranged between 400-460 µmol/g (Maskar *et al.*, 2015). The analysis results of liver MDA and SOD levels were not too different from the previous study. A study by Astawan *et al.* (2015) reported that the liver MDA level was 9.9 µmol/g while liver SOD levels were in the range of 15-40 µmol/g and the level of liver SOD was in the range of 320-410 µmol/g.

In this study, both transgenic and non-transgenic soybean was not known for their parental phenotype, so the chemical component level, as well as the bioactive compounds, could be different. In the USDA database, it was shown that the total soybean isoflavone content from the US was 159.98 mg/100 g of soybean (daidzein level 61.33 mg/100 g, genistein 86.33 mg/100 g, and glycitein 13.33 mg/100 g) (Bhagwat and Haytowitz, 2015). Another factor that affected the increase of the level of MDA was herbicide residue carried in the transgenic crops, so the possibility of *in vivo* cytological alteration occurred. Several studies reported that the provision of soybean ration resulted in negative effects, such as toxicity in the liver organ, especially in the mitochondria (Malatesta *et al.*, 2008; Domingo and Giné Bordonaba, 2011).

The liver organ has a high amount of polyunsaturated fatty acids (PUFA). PUFA can become a precursor of lipid peroxidation if it interacts with reactive oxygen species (ROS) that are highly reactive and unstable. This will inactivate cell apoptosis. ROS is formed due to the influence of the increased oxygen gradient, which causes a decrease in oxygen pressure in the cell and causes oxidative stress (Zainuri *et al.*, 2012). However, other studies stated that experimental rats that consumed soybean ration were not significantly different and did not experience negative effects on the organ of experimental rats (Dixit *et al.*, 2012; Venâncio *et al.*, 2012; Sbruzzi *et al.*, 2013; Himah *et al.*, 2018).

The difference in daily intake also contributes to the tissue modification of experimental animal organs (Romano *et al.*, 2019). The increased protein of soybean flour from 10 to 20% increased the calorie consumption of the experimental rats, which resulted in the increasing weight of experimental rats, as well as an increase in organ weight. The increased protein concentration also increased other chemical compositions in the ration, specifically the fat content. Transgenic soybean flour had the highest fat content compared with other soybean flour types (Table 1), so the PUFA content in transgenic soybean was possibly higher. The high content of fat content in soybean flour can initiate the lipid peroxidation reaction (Dogan and Celik, 2012).

The soaking, heating, and soybean milling during flour production were capable of eliminating anti-nutritional compounds, changing the glycoside isoflavone form into aglycone isoflavones that are readily utilized by the body as an antioxidant, and fragmenting protein and or DNA from foreign genes into smaller forms (Cai *et al.*, 2021; Swallah *et al.*, 2022). Normally, the antioxidants that are available in the body should be sufficient to capture the formed free radicals to create a balanced condition. An excessive number of free radicals will attack the cell macromolecules and cause cell damage or death (Asrin *et al.*, 2018). Soybean isoflavone which functions as an antioxidant has an important role in preventing degenerative diseases such as cancer.

3.3 Level of Cu, Zn-SOD antioxidant in testicular tissue

The immunohistochemical coloring on Cu, Zn-SOD antioxidant levels in the testicular tissue of experimental rats can be seen in Figure 1 and the quantitative observation of Cu, Zn-SOD antioxidant content can be seen in Table 3. The qualitative observation result showed that the casein group had similar Cu, Zn-SOD levels with transgenic, non-transgenic, and local Grobogan soybeans at protein concentrations of 10% and 20%. This is shown by the brown color intensity in a whole similar testicular tissue part (Figure 1).

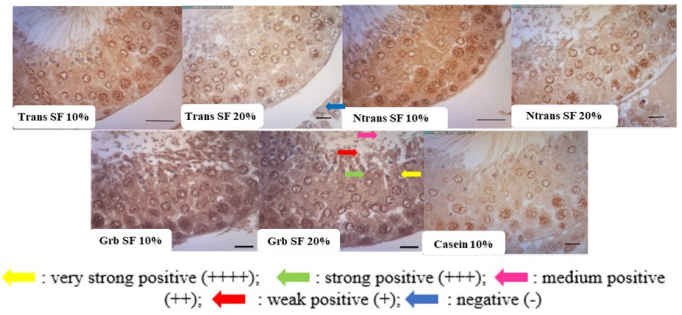

Figure 1. Effect of concentration and protein isolates on the water holding capacity of meatball. Trans SF: Transgenic soy flour, Ntrans SF: Non-transgenic soy flour, Grb SF: Local Grobogan soy flour.

The statistical analysis also showed that commonly the levels of Cu, Zn-SOD in testicular tissue of experimental rats from 10 and 20% transgenic soybean flour and 10 and 20% non-transgenic soybean flour treatments were not significantly different ($p>0.05$) from the casein group. This implied that transgenic and non-transgenic soybean flour treatments with 10 and 20% protein concentration provide similar benefits with 10%

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casein treatment on Cu, Zn-SOD antioxidant content on the testicular tissue of experimental rats. However, 20% transgenic and non-transgenic treatments showed significantly higher results ($p<0.05$) than casein in primary spermatocyte cells which caused a strong positive reaction, early spermatid cells had a strong positive reaction, and for late spermatid, it resulted in a moderately positive reaction. This showed that feeding with protein from soybean flours, namely transgenic, non -transgenic, and even local Grobogan had an equally good result as casein and was effective in increasing the level of Cu, Zn-SOD in testicular tissue of experimental rats. However, 20% transgenic and non-transgenic soybean flour treatment showed significantly higher results ($p<0.05$) than casein in primary spermatocytes which resulted in a very strong positive reaction, early spermatid also had a strong positive reaction, and late spermatid resulted in a moderately positive reaction. This showed that increasing protein concentration affected the increase of spermatogenic cell quantity.

Table 3 also shows that 10 and 20% of local Grobogan soybean treatments overall resulted in significantly different amounts of spermatogenic cells than casein treatment. This could be seen in the number of primary spermatocyte cells which had a strong positive reaction, early spermatid which had a moderately positive reaction, and late spermatid which had a very strong, strong, and moderately positive reaction on 10 and 20% local Grobogan groups that were significantly higher ($p<0.05$) than the casein group.

The result showed that ration that contained protein from soybean flour regardless of transgenic, non-transgenic, and even local Grobogan had similarly better results than casein and was effective in increasing Cu, Zn -SOD in the testicular tissue of experimental rats. The isoflavone acts as an exogenous antioxidant in soybeans and is expected to be capable of preventing oxidative damage and retaining Cu, Zn-SOD antioxidant levels in the testicular tissue. A high level of Cu, Zn-SOD will increase the oxidative defence mechanism, which can increase the spermatogenic cell quantity (Wresdiyati *et al.*, 2018).

The number of spermatogenic cells depends on the seminiferous tubule activity that is regulated by testosterone. If there is a disturbance in the spermatogenesis process due to the decline of the testosterone hormone, the risk of a decline in the quality and quantity of spermatozoa will occur (Carrageta *et al.*, 2022). The combination of soybean isoflavones, Zn, and vitamin E works synergistically in increasing the amount of testosterone and increasing the spermatogenic cells in the seminiferous tubule (Astuti, 2009). Maskar *et al.* (2015) also reported that the provision of transgenic and non-transgenic soybean flour did not negatively affect the quantity of spermatogenic and Leydig cells in experimental rats.

3.4 Spermatozoa profile

The spermatozoa profile analysis was performed on imported (transgenic and non-transgenic) soybean flour at 10 and 20% protein concentration and 10% casein as control. The macroscopic spermatozoa analysis result is shown in Table 4. It showed that color and spermatozoa concentration from the groups which were fed with transgenic and non-transgenic soybean flour was classified as normal with good condition, such as white and thick consistency. The spermatozoa pH of experimental rat groups was also normal.

The microscopic spermatozoa profile analysis result is presented in Table 5. The soybean flour type treatment, protein concentration treatment, and interaction between both did not result in a significant effect ($p>0.05$) in experimental rats for all parameters. However, soybean flour types and protein concentration resulted in significantly lower effects ($p<0.05$) compared with the casein group in the concentration of spermatozoa parameter.

The study result was not too different from the previous study, particularly the abnormality of spermatozoa was in the range of 8-13%, and spermatozoa concentrations were in the range of 1000-1300 cells/mL (Astuti, 2009). The normal motility mass should be between 2-3 scores (good-very good), which is indicated by the active, forward, and fast motion (Iskandar *et al.*, 2006; Savitri *et al.*, 2014). Based on the method by Feradis (2010), the condition of spermatozoa motility in all experimental rat groups was scored as 3 with motility description between 50-80%, and the spermatozoa had progressive movement and mass motion. The abnormality of all groups was also considered normal with a score of <20% (Tolihere, 1985). Isoflavones in the soybean can suppress the abnormality in spermatozoa. Meanwhile, the amino acids act as seminal plasma for spermatozoa formation. The amino acids include aspartate, glutamic acid, and L-arginine, which the presence is needed as a source of energy for normal sperm motility and have roles in the fertility of male rats (Guraya, 2012; Juyena and Stelletta, 2012).

4. Conclusion

Consumption of different soybean flour types and protein concentrations did not significantly affect the SOD level of the kidney and liver and the MDA level in

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Table 3. Number of spermatogenic cells at various levels of Cu, Zn-SOD in experimental rats after 90 days of subchronic test.

Group	Cu, Zn-SOD content level	Number of spermatogenic cells at various levels of Cu, Zn-SOD content in various treatments						
		Casein 10%	Trans SF 10%	Trans SF 20%	Ntrans SF 10%	Ntrans SF 20%	Grb SF 10%	Grb SF 20%
Spermatogonia	++++	11.71±4.96	9.80±0.60	15.60±2.06	9.20±0.60	16.47±2.03	19.54±5.38	19.54±3.61
	+++	14.63±0.32	9.40±0.66	12.27±3.30	10.50±1.02	13.67±2.02	12.81±5.11	14.59±5.79
	++/+	16.02±1.08	16.40±0.77	16.40±2.55	17.70±0.89	19.67±2.82	15.34±3.30	16.54±4.40
	-	0.07±0.12	0.00±0,00	1.47±4.75	0.00±0.00	0.00±0.00	0.00±0.00	0.21±0.42
Primary spermatocytes	++++	8.84±6.25^{a}	3.80±0.60^{a}	18.67±4.98^{ab}	4.20±0.60^{a}	18.87±2.45^{b}	12.08±4.77^{ab}	11.08±6.53^{ab}
	+++	21.93±6.98^{ab}	17.00±1.18^{ab}	19.33±3.26^{ab}	16.40±0.80^{a}	17.60±2.18^{ab}	23.74±8.00^{ab}	24.68±7.61^{b}
	++/+	26.27±7.86	31.70±1.42	20.60±2.06	32.70±0.95	18.60±2.94	25.61±9.71	28.08±6.73
	-	0.39±0.62	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Early spermatids	++++	0.00±0.00	0.00±0.00	0.60±1.78	0.00±0.00	0.13±0.50	0.14±0.53	0.00±0.00
	+++	1.42±1.66^{a}	26.10±1.37^{c}	83.93±8.31^{d}	18.10±1.13^{b}	80.13±7.93^{d}	3.14±5.02^{a}	6.81±7.01^{a}
	++/+	100.87±12.08^{a}	110.30±2.34^{a}	94.73±4.22^{a}	114.70±1.75^{a}	90.40±11.26^{a}	149.61±16.38^{b}	143.54±23.62^{b}
	-	0.30±0.44	0.00±0.00	0.20±0.75	0.00±0.00	0.00±0.00	1.65±5.93	2.35±5.14
Late spermatids	++++	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.34±0.20^{b}	0.68±0.48^{b}
	+++	0.00±0.00^{a}	0.00±0.00^{a}	0.80±1.76^{a}	0.00±0.00^{a}	0.40±0.80^{a}	6.12±3.14^{b}	5.74±2.46^{b}
	++/+	0.75±1.51^{a}	0.00±0.00^{a}	14.53±3.52^{b}	0.00±0.00^{a}	16.27±2.93^{b}	7.74±4.12^{b}	3.99±1.43^{b}
	-	81.77±14.22^{a}	71.40±1.56^{a}	70.80±16.03^{a}	72.40±1.62^{a}	67.67±13.68^{a}	108.01±15.60^{b}	108.36±18.45^{b}

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$) to casein, based on the T-test. Trans SF: Transgenic soy flour, Ntrans SF: Non-transgenic soy flour, Grb SF: Local Grobogan soy flour.

Table 4. Macroscopic profile of spermatozoa in experimental rats after 90 days of subchronic test.

Treatments	Color	pH	Consistency
Trans SF 10%	White	7.1±0.1^{a}	Viscous
Trans SF 20%	White	7.1±0.1^{a}	Viscous
Ntrans SF 10%	White	7.1±0.1^{a}	Viscous
Ntrans SF 20%	White	7.0±0.0^{a}	Viscous
Casein 10%	White	7.0±0.0	Viscous

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$) to casein, based on the T-test. Trans SF: Transgenic soy flour, Ntrans SF: Non-transgenic soy flour.

Table 5. Microscopic profile of spermatozoa in experimental rats after 90 days of subchronic test.

Treatments	Mass movement	Individual movement	Motility (%)	Abnormality (%)	Concentration (10^6 cells/mL)
Trans SF 10%	2.3±0.6[a]	3.0±0.0[a]	76.7±2.9[a]	12.5±8.5[a]	733.3±162.7[a*]
Trans SF 20%	2.3±0.6[a]	2.3±0.6[a]	66.7±7.6[a]	9.8±4.4[a]	1108.3±298.3[a]
Ntrans SF 10%	2.7±0.6[a]	2.7±0.6[a]	68.3±7.6[a]	12.8±4.8[a]	775.0±50.0[a*]
Ntrans SF 20%	3.0±0.0[a]	3.3±0.6[a]	71.7±5.8[a]	8.5±1.1[a]	791.7±94.6[a*]
Casein 10%	3.0±0.0	3.3±0.6	75.0±5.0	7.4±11.1	1241.7±160.7

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$) to casein, based on the T-test. Trans SF: Transgenic soy flour, Ntrans SF: Non-transgenic soy flour, Grb SF: Local Grobogan soy flour.

the kidney but were significantly different in the MDA level in the liver, however, it was still within the safe limit. The administration of transgenic (imported) and non-transgenic (imported and local) soybean flour provided the same benefits as casein on the Cu, Zn-SOD levels in the testicular tissues. Local-Grobogan soybean flour significantly increased the number of spermatogenic cells and Cu, Zn-SOD levels compared to all treatments, indicating the absence of any adverse effect in the testicular tissues of experimental rats.

Conflict of interest

The authors declare no conflict of interest.

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