Clin Nutr Res. 2019 Oct;8(4):296-306
https://doi.org/10.7762/cnr.2019.8.4.296
pISSN 2287-3732·eISSN 2287-3740

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Original Article



# Effects of Isoflavone Supplementation on Lipid Profiles and Antioxidant Enzyme Activities in Growing Rats Fed High Fat Diet

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**Received:** Sep 25, 2019
**Revised:** Oct 15, 2019
**Accepted:** Oct 15, 2019

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**Conflict of Interest**
The authors declare that they have no competing interests.

## ABSTRACT

The purpose of this study was to investigate the effects of isoflavone on serum lipids and antioxidant enzymes activities in growing rats fed high lard diet. Twenty four female Sprague-Dawley rats (body weight 50–60 g) were divided into three groups, control, high fat (HF, lard 200 g/kg diet) and high fat + isoflavone (HFI, lard 200 g/kg diet + isoflavone 310.9 mg/kg diet) for 4 weeks. The results of study indicated that body weight gain was not different by isoflavone diet. Mean intake was significantly lower in HF group and HFI group than control group. Food efficiency ratio was significantly higher in HF group and HFI group than control group. The level of serum triglyceride and total cholesterol were significantly lower in HFI group than control group and HF group. The level of high-density lipoprotein cholesterol, was significantly higher in control group than HF group and HFI group. The level of low-density lipoprotein cholesterol was not significantly different by experimental diets, but atherogenic index (AI) was significantly lower in control group and HFI group than HF group. Contents of total cholesterol and triglyceride in liver tissues were found to be insignificant. The concentration of lipid peroxidation, malondialdehyde was significantly lower in control groups and HFI group than HF group. And antioxidant enzymes in liver tissue were not significantly different by lard and isoflavone supplemented diets. In conclusion, it seems possible that isoflavone supplemented high fat diet may produce positive results on level of serum triglyceride, serum total cholesterol, AI and concentration of malondialdyhyde.

**Keywords:** Isoflavones; Lipids; Antioxidants; Rats; Diet, high fat

## INTRODUCTION

Gradual westernization of the dietary life of people accompanies nutritional imbalance and increased prevalence of diseases in circulatory system such as hypertension due to increasing stress, heart disease, and cerebrovascular diseases. According to the report from the Statistics Korea in 2018, the mortality rate due to heart disease, among three mortal diseases (cancer, cerebrovascular diseases, and heart disease) was 60.2%, which was an increase by approximately 14% compared to that 10 years ago [1]. Hypertension, diabetes mellitus, and hyperlipidemia have been reported to be risk factors of cardiovascular diseases. Favorable dietary control and life-styles away from drinking and smoking can prevent such diseases [2].

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Hyperlipidemia is regarded as one of the risk factors of cardiovascular disease wherein the high level of low-density lipoprotein cholesterol (LDL-C) could cause hypercholesterolemia [3]. Besides, patients suffering from hyperlipidemia are reported with high level of blood cholesterol, high atherogenic index (AI) [4], and level of triglyceride [5]. One study has reported that high fat and high cholesterol diets can induce oxidative damage of internal tissue [6,7]. Enzymes play key roles in the defense system involving antioxidant. They comprise antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT). Dietary natural antioxidants include glutathione, tocopherol, ascorbic acid, carotenoid, and polyphenol compounds [8]. Antioxidant system can lose its balance due to oxidative stress, diseases, and other factors, resulting in an unbalance of the defense mechanism. The creation and promotion of free radicals could damage tissues [9]. Thus, foods selected according to guidelines of diet therapy should be taken to reduce the risk of cardiovascular diseases and improve blood lipid. In particular, total fat level should be considered when taking cholesterol and saturated fat [10].

According to Korea National Health and Nutrition Examination Survey in 2017, 5.2% of the total population had excessive intake of energy/fat. In particular, this percentage increased from 1.5% in 2007 to 5.5% in 2017 for children aged 1–9 years; and from 2.0% in 2007 to 5.0% in 2017 for adolescents of ages 10–18 years [11]. The food sources of fat supply included pork, soybean oil, beef, and so on, with pork accounting for 16.6% as the food source of fat, which was highest among all food sources [12]. Diseases such as hyperlipidemia and cardiovascular disorders can be prevented sufficiently by employing the healthy dietary control and the healthy dietary habit. Therefore, interests in functional foods have been increasing.

Soybean is a food mainly consumed in Asian countries because it is rich in nutrients and diverse functional substances such as isoflavone, saponin, anthocyanin, tocopherol, and phytic acid [13]. Among various substances in soybean, there are aglycones such as daidzein, genistein, and glycitein which are known as vegetative estrogen, and glucosides including daidzin, genistin, and glycitin [14]. According to a recent research, the intake of isoflavone after menopause could increase the amount of calcium in bones of women and prevent osteoporosis [15]. It has been reported that isoflavone can decrease the process of adipogenesis in mouse cells [16]. In addition, the intake of isoflavone can result in improved blood lipid with antioxidant effect in mouse fed with a high cholesterol diet [17]. It can also decrease apoptosis and oxidative stress of female mouse [18]. The effect of intake of isoflavone has been studied previously. It has been reported that isoflavone shows anti-inflammatory and anti-atherosclerosis effects in C57BL/6 mouse [19]. The effect of intake of isoflavone in adult patients suffering hyperlipidemia has also been studied, showing that male patients suffering hyperlipidemia - reduced level of total cholesterol after the intake of isoflavone [20], while adult menopausal women of hyperlipidemia exhibit increased levels of high-density lipoprotein cholesterol (HDL-C) and decreased AI and blood level of malondialdehyde [21].

Although the effect of intake of isoflavone for male and menopausal female adults suffering hyperlipidemia has been studied and reported in terms of improvement in lipids and antioxidant effects, subjects who are in their growth stage requiring higher rate of intake of fat have been hardly employed for studies delving into the effects of the intake of isoflavone to analyze corresponding changes in concentration of lipids and antioxidant indices. Thus, the objective of the present study was to analyze the effect of intake of isoflavone in rats at


https://doi.org/10.7762/cnr.2019.8.4.296

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growth stage fed with high fat to find resulting changes in blood levels of lipids, contents of liver lipids, and antioxidant indices.

## MATERIALS AND METHODS

### Experimental animals and diet

For the experiment conducted in the present study, 28 female Sprague-Dawley rats (50–60 g) were obtained from Daehan Bio-Link Co., Ltd. The entire process covering from adoption to breeding of experimental animals was approved by the Institutional Animal Care and Use Committee of Keimyung University (approval No. KM-2012-35). These experimental animals were acclimated for one week with rat chow (Samyang Co., Seoul, Korea), and then bred by dividing into 3 groups using a completely randomized design. Temperature and humidity of breeding chamber were kept at 22°C ± 2°C and 63% ± 5%, respectively. Periods of light and darkness were controlled to be an interval of 12 hours. Animals were allowed access to diet and water ad libitum. All rats were fed on experimental diet for 4 weeks.

Experimental diet was prepared based on standard compositions of AIN-93G. Dietary compositions of the experimental diet are summarized in **Table 1**. Experimental groups included a control group, a high fat (HF) group, and a high fat + isoflavone (HFI) group in accordance with the addition of high fat and isoflavone into the experimental diet. The amount of lard equivalent to 20% of the amount of experimental diet was added to the basic diet for the HF group, whereas an equal amount of 20% of lard plus 0.1% of isoflavone (total isoflavone 31.09%) was added to the basic diet for the HFI group.

### Measurement of food intake and weight

Weights of experimental animals were measured at an interval of 1 week at predetermined time from the day of the uptake of experimental diet. The amount of food intake was measured at the predetermined time on every other day, and the food efficiency ratio (FER) was calculated by dividing average weight gain of experimental animals by the amount of food intake.

**Table 1.** Composition of experimental diets (g/kg diet)

| Ingredients | Control | HF | HFI |
|---|---|---|---|
| Casein* | 200 | 200 | 200 |
| Corn starch | 529.486 | 329.486 | 328.486 |
| Sucrose | 100 | 100 | 100 |
| Soybean oil | 70 | 70 | 70 |
| α-Cellulose† | 50 | 50 | 50 |
| Min-mixture‡ | 35 | 35 | 35 |
| Vit-mixture§ | 10 | 10 | 10 |
| L-Cystine‖ | 3 | 3 | 3 |
| Choline bitartate¶ | 2.5 | 2.5 | 2.5 |
| TBHQ** | 0.014 | 0.014 | 0.014 |
| Lard†† | - | 200 | 200 |
| Isovone‡‡ | - | - | 1 |
| Kcal (per g diet) | 3.95 | 4.95 | 4.94 |

HF, high fat; HFI, high fat + isoflavone.
*Casein, Maeil dairy industry Co., Ltd., 480 Gagok-Ri, Jinwi-Myun, Pyungtaek-City, Kyunggi-Do, Korea; †α-Cellulose, Sigma Chemical Co., St. Louis, MO, USA; ‡Min-mixture, AIN-93G-Mx, Teklad Test Diets, Madison, Wisconsin, USA; §Vit-mixture, AIN-93G-Vx, Teklad Test Diets, Madison, Wisconsin, USA; ‖L-Cystine, Sigma Chemical Co., St. Louis, MO, USA; ¶Choline bitartate, Sigma Chemical Co., St. Louis, MO, USA; **TBHQ, Aldrich Chemical Co., St. Louis, MO, USA; ††Lard, Chungil Food Co., Ltd. 298 Chujeong-Ri, Chubu-Myeon, Geumsan-Gun, Chungcheongnam-Do; ‡‡Isoflavone, Bioland Co., Ltd. 1058 Shingil-Dong, Danwon-Gu, Ansan-Si, Kyunggi-Do, Korea (Isoflavone component: daidzein 16.55%, glycitein 10.9%. genistein 3.65%, Total isoflavone 31.09%).


https://doi.org/10.7762/cnr.2019.8.4.296

### Preparation of specimens

Experimental animals were anesthetized using ethyl ether. Abdominal incision was made to extract liver and collect blood samples from the aorta. Collected blood samples were left at room temperature for 30 minutes and then centrifuged at 3,000 rpm for 20 minutes to separate serum. To preparation liver specimens, 0.25 M sucrose solution (4 times of the tissue volume) was added and crushed together to produce 20% (w/v) homogenate using a homogenizer. The nuclei and uncrushed parts were removed from the homogenate. The supernatant was then centrifuged at 10,000 ×g (rcf) for 20 minutes. The pellet as mitochondrial fraction and the supernatant as cytosol fraction were obtained to measure activities of enzymes.

### Analysis of lipids in serum

Concentration of cholesterol, triglyceride, and HDL-C in the serum were measured using an Analysis Kit (Asan Pharmaceutical Co., Seoul, Korea). The absorbance was measured using A spectrophotometer (UV-1800, Shimadzu Co., Kyoto, Japan) and then analyzed by colorimetric titration method. The analysis of LDL-C in the serum was performed following method of Friedwald et al. [22]. AI was used to predict the risk of occurrence of atherosclerosis.

### Measurement of malondialdehyde

Malondialdehyde (MDA) in liver tissue was measured using the method described by Ohkawa et al. [23]. For the measurement, 1,1,3,3-tetraethoxypropane (TEP) was employed as the standard material. The level of lipid peroxide(s) is presented as nmole MDA/g.

### Measurement of activity of antioxidant enzymes in liver tissues

To measure the activity of SOD, the hematein, which was created by the methods employed by Martin et al. [24] to observe the degree of suppression of automatic oxidation of hematoxylin, was measured at 560 nm. The activity of GSH-Px was measured according to the method of Paglia and Valentine [25]. Glutathione matrix and nicotinamide adenine dinucleotide phosphate, the coenzyme, were reacted at temperature of 20°C for 5 minutes together with specimens. NADPH is a coenzyme that regenerates glutathione and acts as an electron transporter. The decreasing rate of absorbance owing to oxidation of NADPH was measured at wavelength of 340 nm. The CAT activity of the mitochondrial fraction of the liver tissue was measured according to the method described by Abei [26] which produced the activity by using molecular extinction coefficient by reading absorbance at 240 nm, the degree of reduction, on the matrix of hydrogen peroxide ($H_2O_2$). The content of protein in the liver tissue was measured according to the method employed by Lowry et al. [27] using bovine serum albumin as the standard.

### Statistical analysis

Results obtained from the present study were analyzed using SAS Package (Ver 9.2: Institute Inc., Cary, NC, USA). The mean and standard deviation were obtained for variables. Statistical significance of difference between groups was verified by analysis of variance and Duncan's multiple range test. Significance level was set at $p < 0.05$.


https://doi.org/10.7762/cnr.2019.8.4.296

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## RESULTS

### Body weight, mean intake and FER

Effects of intake of the diet added with isoflavone on weight, mean intake and FER of experimental animals are summarized in **Table 2**. At the beginning of the experiment, the difference in the weight of animals between groups was insignificant (control group 82.4 ± 5.3 g, HF group 82.5 ± 4.2 g, and HFI group 82.6 ± 4.3 g). The final weight of experimental animals in the HF group and HFI group tended to be higher than that of the control group, although intergroup differences were not statistically significant (control group 189.6 ± 14.2 g, HF group 194.2 ± 9.2 g, and HFI group 194.3 ± 12.1 g). Weight gains after the intake of the diet added with isoflavone were not significant among groups (control group 107.1 ± 11.6 g, HF group 111.7 ± 9.7 g, and HFI group 111.7 ± 11.0 g).

The amount of intake of diet appeared to be significantly lower in the HF group and the HFI group than that in the control group (control group 14.8 ± 2.2 g/day, HF group 10.9 ± 0.6 g/day, and HFI group 10.0 ± 0.9 g/day; $p < 0.05$). Efficiency ratio of diet in the HF group or the HFI group was significantly higher than that in the control group (control group 0.26 ± 0.05, HF group 0.36 ± 0.03, and HFI group 0.40 ± 0.06; $p < 0.05$).

### Concentrations of lipids in the serum

Effects of diet added with isoflavone on concentrations of lipids in the serum are summarized in **Table 3**. Concentrations of total cholesterol in the HFI group after the intake of isoflavone appeared to be significantly ($p < 0.05$) lower than that in control group or the HF group (control group 94.5 ± 14.1 mg/dL, HF group 100.3 ± 12.9 mg/dL, and HFI group 84.6 ± 7.6 mg/dL). Concentrations of triglycerides in the HFI group after the intake of isoflavone were significantly ($p < 0.05$) lower than those of the control group and the HF group (control group 125.1 ± 20.5 mg/dL, HF group 128.8 ± 26.4 mg/dL, and HFI group 94.7 ± 8.9 mg/dL). Concentrations of HDL-C in HF group and HFI group were significantly ($p < 0.05$) lower than

**Table 2.** Effects of isoflavone diet on body weight, mean intake and FER in rats fed high fat diet

| Variables | Control | HF | HFI |
|---|---|---|---|
| Initial weight (g) | 82.4 ± 5.3* | 82.5 ± 4.2 | 82.6 ± 4.3 |
| Final weight (g) | 189.6 ± 14.2* | 194.2 ± 9.2 | 194.3 ± 12.1 |
| Weight gain (g) | 107.1 ± 11.6* | 111.7 ± 9.7 | 111.7 ± 11.0 |
| Mean intake (g/day) | 14.8 ± 2.2[a] | 10.9 ± 0.6[b] | 10.0 ± 0.9[b] |
| FER | 0.26 ± 0.05[a] | 0.36 ± 0.03[b] | 0.40 ± 0.06[b] |

Data are presented as mean ± standard deviation.
HF, high fat; HFI, high fat + isoflavone; FER, food efficiency ratio.
[a,b]Values with different superscripts within the row are significantly different at $p < 0.05$ by Duncan's multiple range test. *Not significant.

**Table 3.** Effects of isoflavone diet on serum lipid concentrations in rats fed high fat diet

| Variables | Control | HF | HFI |
|---|---|---|---|
| Total cholesterol (mg/dL) | 94.5 ± 14.13[a] | 100.3 ± 12.9[a] | 84.6 ± 7.6[b] |
| Triglyceride (mg/dL) | 125.1 ± 20.5[a] | 128.8 ± 26.4[a] | 94.7 ± 8.9[b] |
| HDL-C (mg/dL) | 43.6 ± 4.7[a] | 37.7 ± 3.8[b] | 38.0 ± 3.0[b] |
| LDL-C (mg/dL) | 26.4 ± 11.4* | 36.8 ± 12.5 | 27.4 ± 10.2 |
| AI | 1.2 ± 0.3[a] | 1.7 ± 0.4[b] | 1.2 ± 0.3[a] |

Data are presented as mean ± standard deviation.
HF, high fat; HFI, high fat + isoflavone; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; AI, atherogenic index.
[a,b]Values with different superscripts within the row are significantly different at $p < 0.05$ by Duncan's multiple range test. *Not significant.


https://doi.org/10.7762/cnr.2019.8.4.296

those of the control group (control group 43.6 ± 4.7 mg/dL, HF group 37.7 ± 3.8 mg/dL, and HFI group 38.0 ± 3.0 mg/dL). Concentrations of LDL-C after the intake of diet added with or without isoflavone were not significantly different (control group 26.4 ± 11.4 mg/dL, HF group 36.8 ± 12.5 mg/dL, and HFI group 27.4 ± 10.2 mg/dL). AI values of the control group and the HFI group were significantly ($p < 0.05$) lower than those of the HF group after the intake of the diet added with isoflavone (control group 1.2 ± 0.3, HF group 1.7 ± 0.4, and HFI group 1.2 ± 0.3).

## Contents of lipids, malondialdehyde, and activities of antioxidant enzymes in liver tissues

Effects of diet added with isoflavone on contents of lipids, malondialdehyde, and activities of antioxidant enzymes in liver tissues are summarized in **Table 4**. After the intake of the diet added with or without isoflavone, the content of total cholesterol exhibited insignificant intergroup differences as follows: control group 79.3 ± 2.4 mg/g, HF group 81.1 ± 5.3 mg/g, and HFI group 77.0 ± 5.5 mg/g. Similarly, the content of triglyceride after intake of diet added with or without isoflavone revealed insignificant intergroup differences: control group 106.1 ± 17.4 mg/g, HF group 110.2 ± 6.3 mg/g, and HFI group 102.7 ± 14.6 mg/g.

Contents of MDA in the control group and the HFI group were significantly ($p < 0.05$) lower than those in the HF group (control group 1.7 ± 0.3 nmole MDA/g, HF group 2.1 ± 0.1 nmole MDA/g, and HFI group 1.7 ± 0.3 nmole MDA/g).

The activity of SOD in response to the intake of isoflavone manifested insignificant intergroup differences as follows: control group 4.0 ± 1.6 unit/mg protein/min, HF group 3.4 ± 1.6 unit/mg protein/min, and HFI group 3.3 ± 1.6 unit/mg protein/min. The activity of GSH-Px exhibited no significant intergroup difference in response to the intake of isoflavone as follows: control group 4.6 ± 0.4 nmole NADPH/mg protein/min, HF group 4.7 ± 0.7 nmole NADPH/mg protein/min, and HFI group 4.5 ± 1.6 nmole NADPH/mg protein/min. CAT activity manifested no significant intergroup differences either in response to the intake of isoflavone: control group 57.4 ± 16.1 nmole $H_2O_2$ reduced/mg protein/min, HF group 58.0 ± 12.4 nmole $H_2O_2$ reduced/mg protein/min, and HFI group 57.3 ± 13.7 nmole $H_2O_2$ reduced/mg protein/min.

**Table 4.** Effects of isoflavone diet on the contents of lipids, malondialdehyde, and activities of antioxidant enzymes in the liver tissues in rats fed high fat diet

| Variables | Control | HF | HFI |
|---|---|---|---|
| Total cholesterol (mg/g) | 79.3 ± 2.4* | 81.1 ± 5.3 | 77.0 ± 5.5 |
| Triglyceride (mg/g) | 106.1 ± 17.4* | 110.2 ± 6.3 | 102.7 ± 14.6 |
| MDA (nmole MDA/g) | 1.7 ± 0.3[a] | 2.1 ± 0.1[b] | 1.7 ± 0.3[a] |
| SOD (unit/mg protein/min) | 4.0 ± 1.6* | 3.4 ± 1.6 | 3.3 ± 1.6 |
| GSH-Px (nmole NADPH/mg protein/min) | 4.6 ± 0.4* | 4.7 ± 0.7 | 4.5 ± 1.6 |
| CAT (nmole $H_2O_2$ reduced/mg protein/min) | 57.4 ± 16.1* | 58.0 ± 12.4 | 57.3 ± 13.7 |

Data are presented as mean ± standard deviation.
HF, high fat; HFI, high fat + isoflavone; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase.
[a,b]Values with different superscripts within the row are significantly different at $p < 0.05$ by Duncan's multiple range test. *Not significant.


https://doi.org/10.7762/cnr.2019.8.4.296

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## DISCUSSION

The present study intended to identify effects of intake of isoflavone (310.9 mg/kg diet) for rats in growth period fed with high fat diet on in serum lipid concentration, contents of lipids in livers, and antioxidant indices. The amount of high fat added to the diet of 200–220 g of Sprague-Dawley rats was based on a previous study [28], wherein the amount of triglyceride was increased through intake of lard corresponding to 20% of the diet. The amount of isoflavone added to the feed was based on a previous study wherein isoflavone (0.32 g/kg diet) was fed to Sprague-Dawley rats of 210 g which increased enzymatic activity [29].

In the present study, no significant differences in weight gain of animals was found between groups, although the amount of mean intake of feed of the HF group or the HFI group was found to be significantly lower than that of the control group. In addition, FER to be appeared significantly higher in the HF group or the HFI group than that in the control group. However the 2 groups fed with high fat diet showed increases in weight gain despite their lower levels of mean intake of feed. According to a study conducted by Lim et al. [30], when male rats at the age of 4 weeks were fed high fat diet added with isoflavone diet, they showed no significant intergroup difference in weight gain. However, the amount of intake of diet in the group fed high fat diet or the group fed high fat diet + isoflavone appeared to be lower than that of the control group, similar to results of the present study. Another study on the effect of isoflavone on rats fed high fat diet also revealed no intergroup differences in weight gain. However, the group fed with high fat diet, was found to have significantly lower intake of diet compared to the control group [31]. The intake of fat can reduce the amount of dietary unit through its unique flavor together with the sense of satiety [32]. Mitchell et al. [33] have mentioned that the amount of dietary unit can be affected by the preference to feed of subject animals in experiment due to varying compositions therein. In the present study, the level of mean intake of dietary feed was found to be lower in the groups fed with high fat diet. It might be due to effects such as unique flavor of fat and the sense of satiety of the high fat diet.

Concentration of total cholesterol and triglyceride in serum in samples of the HFI group appeared to be significantly lower than those in the control group and the HF group, whereas concentrations of HDL-C in the HF group and the HFI group appeared to be significantly lower than those in the control group. Levels of LDL-C showed no significant intergroup differences, while AI of the control group or the HFI group was significantly lower than that of the HF group. In the meantime, contents of total cholesterol and triglyceride in liver tissues according to the amount of intake of isoflavone were found to be insignificant. According to a study that reported effects of improvement in blood lipids by the intake of isoflavone diet (154.8 mg/kg) for rats fed with lard to induce hyperlipidemia [30], concentrations of total cholesterol and triglyceride in blood and AI of groups fed with high fat added with and isoflavone appeared to be lower than those of the group fed solely fed high fat, similar to results of the present study. In addition, in the study conducted by Liu et al. [34], after male rats (average weight 205.20 g) were fed high fat added with isoflavone (isoflavone 10 mg/kg/day and isoflavone 20 mg/kg/day) for 12 weeks levels of triglycerides in serum samples tended to decrease without showing significant difference. However, levels of total cholesterol in serum samples appeared to be significantly lower in the 2 groups fed with high fat and isoflavone than in the group fed solely with high fat, suggesting an improving effect of intake of isoflavone on total cholesterol level. Besides, Lim et al. [35] have reported that levels of total cholesterol and triglycerides in serum samples are reduced while levels of HDL-C in serum samples were increased in rat with induced hyperlipidemia fed with a diet


https://doi.org/10.7762/cnr.2019.8.4.296

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containing aglycone isoflavone (at 3.5, 7, or 30 g/kg of diet in composition of the feed). Zang et al. [36] took C57BL/6J mice at ages of 6 weeks and fed them with high fat diet added with isoflavone at 0.06%. They reported that consequential AI appeared to decrease. However, the decrease did not affect contents of lipids in the liver, similar to results of the present study. Lee et al. [31] took rats with age of 4 weeks and fed them with beef tallow to determine effects of intake of genistein added to feed by 0.1% and 0.2% of dietary unit. They found that contents of total cholesterol and triglyceride in liver appeared to be lower, contrary to results of the present study. Considering that experimental period lasted 6 weeks in previous studies, meaning that the period of intake of isoflavone sustained longer than the period of the present study, the difference in isoflavone components might have contributed to different results between the present study and previous studies. According to studies conducted by Zhan and Ho [37], the intake of soybean protein containing isoflavone reduced levels of total cholesterol, LDL-C, and triacylglycerol. They suggested that the result might have varied according to contents of isoflavone, period of intake, and sex. Diverse studies on the effect of intake of isoflavones such as genistein and daidzein have been conducted solely or in a complex form. However, the definite mechanism describing the effect of isoflavone clearly is yet to be reported. Thus in-depth studies thereof are required in the future.

Lipid peroxidation is a result of peroxidation reaction such as active oxygen. The level of oxidative stress can be determined by using MDA, the final product of lipid peroxidation which is an indirect indicator identifying the reaction [38]. In the present study, contents of MDA in liver tissues of the control group and the HFI group appeared to be significantly lower than those in the HF group. The contents of MDA in control group and in HFI group decreased by approximately 20% than rats in HF group. According to a study conducted with C57BL/6 mice at age of 5 weeks to determine the antioxidant effects in accordance with the intake of isoflavone, the degree of creation of ThioBarbituric Acid Reactive Substances (TBARS) appeared to be significantly decreased with increasing amount of addition of isoflavone [19]. In the meantime, after rats were fed with high cholesterol and isoflavone by 3 g/kg diet, levels of MDA appeared to be not significantly different from results of the present study. The different effect of the intake of isoflavone might be due to differences in lipid compositions such as cholesterol and triglyceride in the feed [17]. Lim et al. [30] have reported results corresponding to those of the present study, showing that the level of TBARS in serum of rats is decreased in accordance with the intake of isoflavone of rats fed with lard by the level identical to that of the present study (200 g/kg diet). In the present study, anti-atherosclerosis and antioxidant effects in rats fed with high fat were presented indirectly as a consequence of reduced lipid peroxide resulting from the intake of isoflavone. It suggests that the need for soybean foods containing high amount of isoflavone will be increasing in the future.

Regarding activites of SOD, GSH-Px, and CAT as important enzymes in the antioxidant system, they revealed no significant differences in accordance with feeding isoflavone to rats fed with high fat. When the high fat diet containing isoflavone (310.9 mg/kg diet) was fed to experimental animals for 4 weeks, it showed no effect on the antioxidant enzyme system in the present study. Reactive oxygen species (ROS) can affect internal homoeostasis, trigger oxidative stress [39], and cause peroxidation of lipids. It has been reported that ROS could cause diseases such as diabetes mellitus and atherosclerosis [40]. Vidal et al. [41] have reported higher level of intake of fat is associated with higher level of stress, suggesting that factors, such as diseases besides oxidative stress, can create peroxides and cause damages to tissues, thus affecting the antioxidant system [9]. SOD, CAT, and GSH-Px are important antioxidant enzymes that can remove ROS through mutual interactions. SOD converts


https://doi.org/10.7762/cnr.2019.8.4.296

peroxy-radical ($O_{2-}$) into hydrogen peroxide wherein catalase converts hydrogen peroxide into water and oxygen. GSH-Px also transforms hydrogen peroxide into water and shows antioxidant reactions by removing radicals [25,26]. According to the study that analyzed the antioxidant index of rats fed with isoflavone and a high fat diet [42], rats fed with high fat showed no significant intergroup differences in GSH-Px according to the intake of isoflavone (genistein 320 mg/kg diet), whereas the activity of catalase showed significant increase in the group fed with isoflavone and high fat that in the group fed with high fat solely, different from results of the present study. The difference might be ascribable to different ages (in weeks), sex, and diets of experimental animals between the 2 studies. In the previous study, male adult rats of 210 g in weight were used together with the diet of high fat employing beef tallow. Lee et al. [29] have conducted a test employing male rats aged 7 weeks and fed them with high fat to determine the effect of intake of isoflavone (320 mg/kg diet) for 10 weeks. They found that the activity of antioxidant enzyme in the liver showed no significant intergroup differences, although the level of erythrocytes and activities of catalase and GSH-Px in the group fed with high fat and isoflavone appeared to be higher than those of the group solely fed with high fat. They suggested that the decrease in oxidative stress in blood and liver was due to the intake of isoflavone. In the present study, liver MDA contents in the group of rats fed with high fat and isoflavone diet were decreased without affecting activities of antioxidant enzymes. The unaffected activities of antioxidant enzymes might be attributable to the short period of experiment conducted in the present study. Thus, further studies delving into the identification of effective internal synergism of the intake of equivalent amount of isoflavone for a longer period of time, are warranted.

## CONCLUSION

In conclusion, the intake of isoflavone provides beneficial effect on metabolism of lipid peroxide by decreasing MDA content in tissues of liver, and improved the serum status of total cholesterol and triglyceride together with decrease in AI for the growing rat which were fed with high fat feed.

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https://doi.org/10.7762/cnr.2019.8.4.296