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# Effects of Isoflavones Supplementation on Cholesterol and Fatty Acid Levels of Muscle and Liver Tissues of Quail

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**Abstract:** Dietary soy isoflavones reduce hepatic 3-hydroxy-3-methylglutaryl CoA reductase and Delta six desaturase activities and isoflavone metabolites from diet may act as scavengers of reactive oxygen species. Since, dietary intake of soy isoflavones has been associated with reduced lipid perxidation, we investigated the effects of soy isoflavone supplementation on the cholesterol and fatty acid levels of muscle and liver tissue in Japanese quails (*Coturnix coturnix japonica*). One hundred and twenty quail (5 month old) were assigned to three experimental groups consisting of 10 birds in each group. Birds were fed either a basal diet or a basal diet supplemented with 200 or 800 mg of soy isoflavones/kg of diet. The animals were sacrificed after 10 month and the tissue samples werecollecetd and analyzed in HPLC and GC equipments. Muscle and liver fatty acid composition, palmitic acid (16:0), palmitoleic acid (16:1, n-9), oleic acid (18:1, n-9), linoleic (18:2, n-6) and linolenic acids (18:3, n-3) increased ($p<0.001$), whereas, stearic acid (18:0) ($p<0.001$) eicosatrienoic (20:3, n-6), arachidonic (20:4, n-6) and eicosapentaenoic acids (20:5, n-3) docosahexaenoic acid level (226, n-3) concentrations decreased with soy isoflavone supplementation. Liver and muscle cholesterol concentrations decreased decreased with soy isoflavone supplementation ($p<0.001$). As a result, administration of isoflavones was seen to elevate the amount of monounsaturated fatty acids in both muscle and liver tissues, while, reducing the amount of polyunsaturated fatty acids. This suggests that isoflavones influence the liver enzymes taking part in the lipid metabolism.

**Key words:** Isoflavone, cholesterol, fatty acid composition, liver, muscle

## INTRODUCTION

Fatty acids affect many structural, metabolic and regulatory component of cells (Jump, 2004). Cholesterol has long been acknowledged to play a key role in determining eukaryotic membrane structure and dynamics, allowing regulation of integral membrane protein activity through composition, in the essentially isothermal/ isobaric environment of the cell (Pitman and Suits, 2004). As essential amino acids and fatty acids cannot be synthesized by animals and humans, they are taken from foods and have to be converted within the metabolism (Jiang *et al*., 1998). Isoflavones, called phytoestrogens, which are compounds of sterol origin, are produced from plants (Anderson and Garner, 1997; Sirtori, 2001). Isoflavones, which constitute a significant phytochemical for animals and humans, are found in high amounts in the soy plant (Faraj and Vasanthan, 2004). The soy plant contains daidzein and genistein isoflavones most (Barnes and Kim, 1998). These compounds are found in the soybean either as glucosides or in free form as glucans (Coward *et al*., 1998). Genistein is converted first into a glycone form and then to dehydrogenistein and p-ethylphenol by colonic microflora. The other major isoflavone of the soy, daidzein, is converted into O-desmethylangolensin and dihydrodaidzein after hydrolysis (Heinonen *et al*., 1999). Isoflavones, which are found abundantly in the soybean and which are a group of flavonoids, have been established to have very high biological activities (Sonee *et al*., 2004; Murphy and Hendrich, 2002). The effects of the consumption of isoflavones obtained from soy products on health have been investigated (Umphress *et al*., 2005). There are findings indicating that these phytochemicals are effective in preventing heart diseases and diabetes (Wang *et al*., 2002), lead to a decline in the incidence of

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such cancer types as breast, prostate and colon cancer (Birt *et al*., 2001) show strong antioxidant effects in preventing lipid peroxidation (Santiago *et al*., 1992) and reduce the rate of Low-Density Lipoprotein (LDL).

There are only a few studies about the effect of isoflavones on quails. It was reported that phytoestrogens caused a decrease in the reproduction of California quails (Leopold *et al*., 1976; Fitzpatrick, 2003). Similarly, it was stated that the number of copulations dropped in Japanese quails, which were administered different doses of genistein (Panzica *et al*., 2005). The present study was to investigate the effects of dietary soy isoflavones on fatty acids composition and cholesterol values in liver and muscle tissues of quail.

## MATERIALS AND METHODS

**Animal selection and treatment:** One hundred and twenty, 5 month old Japanese quails (*Coturnix coturnix japonica*) were used in this study. The birds were assigned randomly to three treatment groups consisting 10 birds each group. The birds were fed a basal diet in the control group containing 17% Crude Protein (CP) and 12.4 MJ $kg^{-1}$ Metabolizable Energy (ME) whereas the birds in the two treatment groups were fed basal diet or basal diet supplemented with either 200 or 800 mg of soy isoflavones/kg of diet. Soy isoflavone supplement was provided by Solgar Inc., Istanbul, Turkey. Water and diets were offered ad libitum throughout the experiment. The bird-house was lit for 17 h $day^{-1}$. At the end of 10 months, birds were slaughtered and muscle and liver tissues were collected. These tissue parts were washed immediately in 0.9% serum physiologic to rinse off blood.

**Total cholesterol analysis:** Tissue samples (500 mg) were extracted in 3 mL acetonitrile/isopropyl alcohol (7030, v $v^{-1}$)-containing tubes and the mixture were vortexed for 30 sec and centrifuged at 6000×g for 10 min at 4°C. Supernatants were transferred to autosampler vials of the HPLC instrument. Acetonitrile-isopropyl alcohol (7030 v $v^{-1}$) was used as mobile phase at a flow rate of 1 mL $min^{-1}$ (Katsanidis and Addis, 1999). Supelcosil LC 18™ DB column (250×4.6 mm, 5 μm) was used as the HPLC column. Detection was performed by UV at 202 nm and 40°C column oven (Bragagnolo and Rodriguez-Amaya, 2003). Quantification was carried out by external standardization using Class VP software. The results were expressed as μmol $g^{-}$ wet tissue samples.

**Extraction of lipids, preparation of fatty acid methyl esters and Gas Chromatographic (GC) analyses:** Extraction of lipids from the tissue samples was performed according to Hara and Radin (1978) method, which uses 32 (v $v^{-1}$) hexane isopropanol mixtures. According to this method, 1 g liver and muscle tissue was broken down in a Micra-D.8 homogeniser at 11.000 rpm for 1 min with 10 mL hexane isopropanol mixture having a rate of 32 (v $v^{-1}$). The tissue homogenate was put into 15 mL centrifuge tubes and centrifuged at 15.000×g to separate the tissue pellet. The supernatant part was put into capped tubes and stored at -25°C until a second analysis. The organic solvent in which the tissues were broken down was added 0.01% butylated hydroxytoluene (BHT). Fatty acid methyl esters were prepared according to Christie (1990) methylation method. After the fatty acids in the lipid extract were converted into methyl esters, they were analyzed in SHIMADZU GC 17 ver.3 gas chromatography. A 25 cm long MACHERY-NAGEL (Germany) capillary colon with a 0.25 μm internal diameter and a PERMABOND 25 micron film thickness was used in this analysis. Throughout the analysis, colon temperature was kept at 120-220°C, injection temperature at 240°C and detector temperature at 280°C. Nitrogen was used as the carrier gas. Mixtures of standard fatty acid methyl esters were injected to determine the retention time of each fatty acid. Following this procedure, appropriate programming was conducted and the fatty acid methyl ester mixtures of the samples were analyzed.

**Statistical analyses:** The data was reported as mean±SEM. Statistical analysis was performed using SPSS Software (Version 10). Analysis of variance (ANOVA) and an LSD test were used to compare the experimental groups with the controls.

## RESULTS

**Cholesterol concentrations of muscle and liver:** Although, amount of cholesterol in the muscle tissue was not different in the group administered 200 mg isoflavone and control group, it was found to be lower in the group administered 800 mg isoflavone, in comparison to the control group (Table 1) ($p<0.001$). Amount of cholesterol in the liver tissue was found to have declined in both isoflavone groups (Table 1) ($p<0.001$).

**Fatty acid composition of muscle and liver:** Analyses of the fatty acids obtained from the muscle tissue revealed that palmitic acid (16:0) elevated in isoflavone groups,

Table 1: The effecets of soy isoflavones on cholestrol concentrations of muscle and liver tissues (μg $g^{-1}$ tissue)

| | Control | 200 | 800 |
|---|---|---|---|
| Liver | 431.31±15.07$^{a}$ | 318.61±7.27$^{c}$ | 359.08±20.80$^{b}$ |
| Muscle | 184.97±6.74$^{a}$ | 197.18±5.11$^{a}$ | 121.84±9.95$^{c}$ |

a-c: Different letters indicate significant differences between groups ($p<0.05$)

Table 2: The effecets of soy isoflavones on fatty acid composotion of muscle tissue (%)

| Fatty acids | Control | 200 | 800 |
|---|---|---|---|
| 14:0 | 0.25±0.02 | 0.51±0.03 | 0.43±0.02 |
| 16:0 | 17.50±0.62$^{b}$ | 21.07±0.31$^{a}$ | 19.78±0.64$^{a}$ |
| 16:1, n-7 | 3.96±0.38$^{b}$ | 5.16±0.32$^{a}$ | 4.97±0.32$^{a}$ |
| 18:0 | 18.31±0.60$^{a}$ | 11.42±0.48$^{b}$ | 12.13±1.02$^{b}$ |
| 18:1, n-9 | 16.50±1.23$^{b}$ | 27.44±1.09$^{a}$ | 26.68±1.41$^{a}$ |
| 18:2, n-6 | 20.33±0.34$^{b}$ | 20.45±0.37$^{b}$ | 22.21±0.64$^{a}$ |
| 18:3, n-3 | 0.65±0.06 | 0.82±0.05 | 0.92±0.07 |
| 20:3, n-6 | 0.71±0.02 | 0.18±0.03 | 0.12±0.007 |
| 20:4, n-6 | 11.14±0.64$^{a}$ | 6.38±0.36$^{b}$ | 6.19±0.59$^{b}$ |
| 20:5, n-3 | 0.56±0.03 | 0.26±0.03 | 0.28±0.03 |
| 22:4, n-6 | 0.47±0.06 | 0.77±0.06 | 0.68±0.07 |
| 22:5, n-6 | 0.58±0.01 | 0.40±0.02 | 0.38±0.03 |
| 22:5, n-3 | 0.76±0.07 | 0.49±0.06 | 0.43±0.04 |
| 22:6, n-3 | 8.36±0.77$^{a}$ | 4.89±0.30$^{b}$ | 4.80±0.56$^{b}$ |
| Σ Saturated | 36.06±0.43 | 33.00±0.26 | 32.34±0.53 |
| Σ Unsaturated | 63.64±0.30 | 67.24±0.27 | 68.18±0.32 |
| Σ MUFA[1] | 20.46±0.76 | 32.60±0.76 | 31.65±0.82 |
| Σ PUFA[2] | 43.16± 0.25 | 34.64±0.17 | 36.53±0.26 |
| Σ ω-3 | 10.33±0.24 | 6.43±0.13 | 6.15±0.19 |
| Σ ω-6 | 33.23±0.22 | 28.21±0.18 | 30.38±0.30 |

a-b: Different letters indicate significant differences between groups ($p<0.05$); MUFA[1]: Monounsaturated Fatty Acid; PUFA[2]: Polyunsaturated Fatty Acid

Table 3: The effecets of soy isoflavones on fatty acid composotion of liver tissue (%)

| Fatty acids | Control | 200 | 800 |
|---|---|---|---|
| 14:0 | 0.59±0.04 | 0.61±0.05 | 0.48±0.02 |
| 16:0 | 26.60±0.63$^{a}$ | 27.13±0.32$^{a}$ | 25.22±0.67$^{b}$ |
| 16:1, n-9 | 4.09±0.16 | 4.38±0.13 | 3.73±0.10 |
| 18:0 | 18.47±0.54$^{a}$ | 16.89±0.57$^{b}$ | 18.62±0.28$^{a}$ |
| 18:1, n-9 | 19.07±0.43$^{b}$ | 27.08±0.72$^{a}$ | 19.97±0.24$^{b}$ |
| 18:2, n-6 | 14.40±0.34$^{a}$ | 11.31±0.34$^{b}$ | 14.72±0.67$^{a}$ |
| 18:3, n-3 | 0.65±0.05 | 0.41±0.11 | 0.50±0.05 |
| 20:2, n6 | 0.36±0.03 | 0.32±0.09 | 0.32±0.03 |
| 20:3, n-6 | 0.52±0.04 | 0.36±0.04 | 0.49±0.03 |
| 20:4, n-6 | 7.22±0.24$^{a}$ | 5.31±0.39$^{b}$ | 7.33±0.29$^{a}$ |
| 20:5, n-3 | 0.51±0.03 | 0.36±0.07 | 0.60±0.12 |
| 22:4, n-6 | 0.58±0.06 | 0.64±0.11 | 0.69±0.07 |
| 22:5, n-6 | 0.21±0.03 | 0.23±0.02 | 0.26±0.08 |
| 22:5, n-3 | 0.43±0.06 | 0.30±0.06 | 0.42±0.04 |
| 22:6, n-3 | 5.90±0.28 | 4.6700±0.31 | 6.74±0.22 |
| Σ Saturated | 45.66±0.45 | 44.63±0.36 | 44.32±0.35 |
| Σ Unsaturated | 53.87±0.15 | 54.96±0.18 | 55.77±0.14 |
| Σ MUFA[1] | 23.16±.30 | 31.46±0.41 | 23.70±0.18 |
| Σ PUFA[2] | 30.71±0.10 | 23.50±0.16 | 32.07±0.18 |
| Σ ω-3 | 7.48±0.11 | 5.27±0.12 | 7.66±0.11 |
| Σ ω-6 | 23.23±0.14 | 18.13±0.18 | 24.41±0.17 |

a-b: Different letters indicate significant differences between groups ($p<0.05$); MUFA[1]: Monounsaturated Fatty Acid; PUFA [2]: Polyunsaturated Fatty Acid

relative to the control group ($p<0.001$). Amount of palmitoleic acid (16:1, n-9) increased in the 200 mg isoflavone group ($p<0.03$), but did not change in 800 mg isoflavone group, compared to the control group ($p>0.05$). Stearic acid (18:0) declined in both isoflavone doses, whereas oleic acid (18:1, n-9) increased ($p<0.001$). Amount of linoleic acid (18:2, n-6) did not change in the 200 mg isoflavone group ($p>0.05$), but increased in the 800 mg isoflavone group, relative to the control group ($p<0.01$). Similar results were obtained with linolenic acid (18:3, n-3), which was observed to increase in the 800 mg isoflavone group, in comparison to the control group ($p<0.005$). Eicosatrienoic acid (20:3, n-6), arachidonic acid (20:4, n-6) and eicosapentaenoic acid (20:5, n-3) were observed to fall in isoflavone-administered groups, relative to the control group ($p<0.001$). It was found that amount of docosatetraenoic acid increased in isoflavone groups, in comparison to the control group ($p<0.003$), while, docosapentaenoic acid (22:5, n-6) did not change ($p>0.05$). Docosapentaenoic acid (22:5, n-3) did not change in the 200 mg isoflavone group ($p>0.05$), but declined in 800 mg isoflavone group, relative to the control group ($p<0.001$). Amount of docosahexaenoic acid (22:6, n-3) was found to decrease in isoflavone administered groups, when compared to the control group ($p<0.001$) (Table 2).

It was found that myristic acid (14:0), palmitic acid (16:0) and palmitoleic acid (16:1, n-7) in the liver tissue did not vary among groups ($p>0.05$). Stearic acid (18:0) value dropped in 200 mg isoflavone group ($p<0.05$), but did not change in 800 mg isoflavone group ($p>0.05$). Oleic acid (18:1, n-9) was found to increase in 200 mg isoflavone group relative to the control group ($p<0.001$), but did not change in the 800 mg isoflavone group ($p>0.05$). Amounts of linoleic (18:2, n-6), linolenic (18:3, n-3), eicosatrienoic (20:3, n-6), arachidonic (20:4, n-6) and docosahexaenoic acid (22:6, n-3) decreased in 200 mg isoflavone group ($p = 0.05$), but did not change in the group that was administered 800 mg isoflavone ($p>0.05$). No difference was observed in the amounts of eicosapentaenoic (20:5, n-3), docosatetraenoic (22:4, n-6) and docosapentaenoic acid (22:5, n-3) between the control group and isoflavone groups ($p>0.05$) (Table 3).

## DISCUSSION

Diets rich in isoflavones influence several biochemical values in both humans and animals (Potter *et al*., 1993; Baum *et al*., 1998; Crouse *et al*. 1999; Farhan *et al*., 2002). Including lipid metabolism, which is important in many diseases. The soy protein was shown to affect the lipid metabolism by influencing the activities of sterol-regulatory element binding proteins (SREBP) (Torres *et al*., 2006). Similarly, intake of soy protein was demonstrated to reduce expression of fatty acid synthase (FAS), malic enzyme, storage of triglycerides in the liver and the amount of cholesterol (Ascencio *et al*., 2004). In the present study, amount of cholesterol in muscle tissue of birds did not change in the group administered 200 mg isoflavone, but decreased in the group that was administered 800 mg isoflavone. Amount of cholesterol in the liver tissue of the quails, on the other hand, was found to decrease in both doses of isoflavone (Table 1). Similar to our results, it is reported that isoflavones have been implicated as contributors to its hypocholesterolaemic

effect (Crouse *et al*., 1999; Ascencio *et al*., 2004). It was recently demonstrated in an isoflavone dose-response study that a daily intake of 25 g of soy protein containing 37-62 mg of isoflavones could significantly lower total and LDL cholesterol. It was found that dietary soy isoflavones lowered hepatic 3-hydroxy-3-methylglutaryl CoA reductase activity, although, liver cholesterol level was not modulated in rats (Anthony *et al*., 1996). However, the levels of serum cholesterol and triglyceride decreased by consumption of soy isoflavones. Therefore, dietary soy isoflavones may exhibit hypocholesterolmic and hypolipidemic functions (Kawakami *et al*., 2004).

The other main component of the lipid metabolism is the types of fatty acids. Of these, fatty acids like myristic (14:0), palmitic (16:0), palmitoleic (16:1, n-7), stearic (18:0), oleic (18:1, n-9) acids are synthesized endogenously in all the animal tissues (Legrand and Bensadoun 1991; Leonarda *et al*., 2004). The synthesis of 16:1 from 16:0 is realised via introduction of a double bond between the 9th and 10th Cs by Delta 9 desaturase (Steroil CoA desaturase) enzyme. Activities of both the fatty acid synthetase (FAS) and delta 9 desaturase enzymes are affected by different diets, various hormones and dietary supplements (Ntambi, 1999; Rimoldi *et al*., 2001). In this study, amount of palmitic acid was observed to increase in the muscle tissue in both isoflavone groups, but did not change in the liver tissue. Likewise, stearic acid amount declined, while, oleic acid amount increased in the muscle tissue of both groups which were administered isoflavone, relative to the control group. It is assumed that the decrease in stearic acid and the increase in oleic acid in the liver tissues of the group that was administered 200 mg isoflavone may have resulted from the increase in the activity of Delta 9 desaturase enzyme. Since, muscle tissue lacks the capability of de novo synthesizing fatty acid (FA), myocytes rely on the supply of FA from extracellular sources to cover their need of these substrates (Van Bilsen *et al*., 1997). Since, the metabolic fate of FA in skeletal muscle may depend on chain length and degree of unsaturation (Van der Vusse *et al*., 1998). It is of importance to analyze the relative composition of the FA pool in these tissues. FA not only substantially contribute to muscle oxidative energy conversion, but also serve as substrates for biological active compounds, such as eicosanoids and membrane phospholipid synthesis and act as ligands for protein factors involved in signalling transduction and gene expression.

Lipid metabolism in the liver is very complex and involves, among many other functions, the synthesis and secretion of very low density lipoproteins and a high rate of fatty acid oxidation, which provides most of the energy that the organ requires to exert its many functions in the organism (Fla´via and Mari´lia, 2004).

This fatty acid metabolism starts with linoleic (18:2, n-6) and linolenic (18:3, n-3) acids. Synthesis of such acids as stearidonic (18:4, n-3), eicosadienoic (20:2), eicosatrienoic (20:3, n-6), arachidonic (20:4, n-6), eicosapentaenoic (20:5, n-3), docosapentaenoic (22:4, n-6), docosapentaenoic (22:5, n-3) and docosahexaenoic (22:6, n-3) are synthesized in the animal tissues via Delta 6 desaturation, called Delta 6 and 5 desaturase enzymes (Leonarda *et al*., 2004; Ntambi, 1999; Cheul Kim and Nitambi, 1999). According to the results of our study, excessive amounts of polyunsaturated fatty acids in the muscle tissue were observed to decrease in both isoflavone groups in comparison to the control group, while, such a decrease was found in the liver tissue only in the group which was administered 200 mg isoflavone. Kawakami *et al*. (2004) was observed that the dietary soy isoflavones lowered hepatic delta 6 desaturase activity in rats. Reflecting Kawakami *et al*. (2004) observation, Delta 6 desaturation I ndices linoleic (18:2, n-6), linolenic (18:3, n-6) and arachidonic (20:4, n-6) acids of tissue lipids tended to be lower in rats fed isoflavones than in those fed isoflavones free diet. According to results, it was suggested that the prevention of inflammatory response by imbalance of eicosenoids may be contributed. Isoflavone glucosides such as puerarin and daidzein have showed lower inhibitory activities on the release of arachidonic acid and metabolites (Jun *et al*., 2005).

It has been noted that liver fatty acid metabolism is controlled by such enzymes as ATP citrate-lyase (ACL), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), malic enzyme (ME) and stearoyl-CoA desaturase 1 (SCD-1), which functions in fatty acid desaturation and secretion (Towle *et al*., 1996; Daval *et al*., 2000). Different food and hormones influence the genes of these enzymes (Jump and Clarke, 1999). Furthermore, SREBP-1 regulates the expression of SCD-1, delta 6 and 5 desaturase enzymes, which are necessary for polyunsaturated and monosaturated fatty acid formation (Assaf *et al*., 2004; Dobrzyn and Ntambi, 2005). Hepatic SCD-1 activity affects fatty acid synthesis, triglyceride esterification and amount of very low-density lipoprotein (VLDL) cholesterol (Ntambi *et al*., 2002; Tovar *et al*., 2005). Decrease in SCD-1 in the livers of rats fed on soy protein is noted as an explanatory mechanism for the decrease in VLDL and hepatic triglyceride in circulation (Tovar *et al*., 2005). In our study, it was observed that both cholesterol and some fatty acid values changed in both muscle and liver tissues of Japanese quails. It is believed that this change has been caused by the effect isoflavone administration has on the activities of enzymes functioning in the lipid metabolism of liver. In addition, these observations may contrubute to the antiin-

flammatory properties of isoflavones and may reduce he risk of some cardiovascular diseases through their radical scavenging function and hypocholestero-lemic action.

## REFERENCES


Anderson, J. and S. Garner, 1997. Phytoestrogens and human function. Nutr. Today, 32: 232-239.

Anthony, MS., T.B. Clarkson, C.L. Hughes, T.M. Morgan and G.L. Burke, 1996. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J. Nutr., 126: 43-50.

Ascencio, C., N. Torres, F. Isoard-Acosta, F.J. Gómez-Pérez, R. Hernández-Pando and A.R. Tovar, 2004. Soy protein affects serum insulin and hepatic SREBP-1 mRNA and reduces fatty liver in rats. J. Nut., 134 (3): 522-529.

Assaf, S., S. Lagarrigue, S. Daval, M. Sansom, B. Leclercq, J. Michel, F. Pitel, M. Alizadeh, A. Vignal and M. Douaire, 2004. Genetic linkage and expression analysis of SREBP and lipogenic genes in fat and lean chicken. Comparative Biochemistry and Physiology. Part B. Biochem. Mol. Biol., 137 (4): 433-441.

Barnes, S. and H. Kim, 1998. Soy isoflavones, estrogens and growth factor signaling. Soy Connec. Newslett., 6: 2.

Baum, A., H. Teng, Jr, J.W. Erdman, R.M. Weigel, B.P. Klein, V.W. Persky, S. Freels, P. Surya, R.M. Bakhit, E. Ramos, N.F. Shay and S.M. Potter, 1998. Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-lipoprotein receptor messenger RNA in hypercholes-terolemic, postmenopausal women. Am. J. Clin. Nutr., 68: 545-551.

Birt, D.F., S. Hendrich and W. Wong, 2001, Dietary agents in canser prevention Flavonoids and isoflavanoids. Pharmancol. Therapeut., 90: 157-177.

Bragagnolo, N. and D.B. Rodriguez-Amaya, 2003. Comparison of the cholesterol content of Brazilian chicken and quail eggs. J. Food Comp. Anal., 16 (2): 147-153.

Cheul Kim, Y. and M. Nitambi, 1999. Regulation of Stearoyl-CoA desaturase genes; role in cellular metabolism and preadipocyte differentiation. Biochem. Biophys. Res. Commun., 266: 1-4.

Christie, W.W., 1990. Gas Chromatography and Lipids, 302. The Oil Press, Glaskow.

Coward, L., M. Smith, M. Kirk and S. Barnes, 1998. Chemical Modification of İsoflavones in soy foods during coduing and processing. Am. J. Clin. Nutr., 68: 14865-14915.

Crouse, III, J.R., T. Morgan, J. Terry, J. Ellis, M. Vitolins and G.L. Burke, 1999. A randomized trial comparing the effect of casein with that of soy protein containing varying amounts of isoflavones on plasma concentrations of lipids and lipoproteins. Arch. Int. Med., 159: 2070-2076.

Daval, S., S. Lagarrigue and M. Douaire, 2000. Messenger RNA levels and transcriptional rates of hepatic lipogenic genes in genetically lean and fat chickens. Gene. Selec. Evolu., 32: 521-531.

Dobrzyn, A. and J.M. Ntambi, 2005. The role of stearoyl-CoA desaturase in the control of metabolism. Prostaglandins Leukot Essent Fatty Acids, 73: 35-41.

Faraj, A. and T. Vasanthan, 2004. Soybean isoflavones effects of processing and health benefits. Food Rev. Int., 20: 51-75.

Farhan, H., K. Wahala, H. Adlercreutz and H.S. Cross, 2002. Isoflavonoids inhibit catabolism of vitamin D in prostate cancer cells. J. Chromatogr. B., 777: 261-268.

Fitzpatrick, L.A., 2003. Soy isoflavones hope or hype?. Maturitas, 44 (1): 21-29.

Fla´via R. Carren'o and C.L. Mari´lia, 2004. Seelaender Liver denervation affects hepatocyte mitochondrial fatty transport capacity. Cell Biochem. Funct., 22: 9-17.

Hara, A. and N.S. Radin, 1978. Lipid extraction of tissues with a low-toxicity solvent. Analiti. Biochemist., 90 (1): 420-426.

Heinonen, S., K. Wahala and H. Adlercreutz, 1999. Identification of isoflavone metabolites dihydro-daidzein, dihydrogenistein, 6'-OH-O-dma and cis-4-OH-equol in human urine by gas chromatography-mass spectrometry using authentic reference compounds. Analytical. Biochemist., 274: 211-219.

Jiang, W.G., R.P. Bryce and D.F. Horrobin, 1998. Essential fatty acids molecular and cellular basis of their anti-cancer action and clinical implications. Critic. Rev. Oncol. Hematol., 27: 179-209.

Jump, D.B., 2004. Fatty acid regulation of gene transcription. Crit. Rev. Clin. Lab. Sci., 41 (1): 41-78.

Jump, D.B. and S.D. Clarke, 1999. Regulation of gene expression by dietary fat. Ann. Rev. Nutr., 19: 63-90.

Jun, M., J.G. Hong, W.S. Jeong and C.T. Ho, 2005. Suppression of arachidonic acid metabolism and nitric oxide formation by kudzu isoflavones in murine. Mol. Nutr. Food Res., 49 (12): 1154-1159.

Katsanidis, E. and P.B. Addis, 1999. Novel HPLC analysis of tocopherols and cholesterol in tissue. Free Radic. Biol. Med., 27 (11-12): 1137-1140.

Kawakami, K., W. Tsurugasaki, Y. Yoshida, Y. Igarashi, S. Nakamura and S. Osada, 2004. Regulative actions of dietary soy isoflavone on biological antioxidative system and lipid metabolism in rats. J. Agric. Food Chemist., 52 (86): 1764-1768.

Legrand, P. and A. Bensadoun, 1991. Stearoyl-CoA desaturase activity in cultured rat hepatocytes. Biochimica et Biophysica Acta, 1086: 89-94.

Leonarda, A.E., S.L. Pereiraa, H. Sprecherb and Y.S. Huangam, 2004. Elongation of long-chain fatty acids. Progress Lipid Res., 43: 36-54.

Leopold, A.S., M. Erwin, J. Oh and B. Browning, 1976. Phytoestrogens Adverse effects on reproduction in California quail. Science, 191: 98-100.

Murphy, P.A. and S. Hendrich, 2002. Phytoestrogens in foods. Adv. Food Nutr. Res., 44: 195-246.

Ntambi, J.M., M. Miyazaki, J.P. Stoehr, H. Lan, C.M. Kendziorski and B.S. Yandell, 2002. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. In: Proc. Nation. Acad. Sci. United States Am., 99: 11482-11486.

Ntambi, J.M., 1999. Regulations of Stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J. Lipid Res., 409: 1549-1558.

Panzica, G.C., E. Mura, M. Pessatti and C. Viglietti-Panzica, 2005. Early embryonic administration of xenoestrogens alters vasotocin system and male sexual behavior of the Japanese quail. Domest. Anim. Endocrinol., 29 (2): 436-445.

Pitman, M.C. and F. Suits, 2004. Molecular-Level Organization of Saturated and Polyunsaturated Fatty Acids in a Phosphatidylcholine Bilayer Containing Cholesterol. Biochemistry, 43: 15318-15328.

Potter, M., R.M. Bakhit, D.L. Essex-Sorlie, K.E. Weingartner, K.M. Chapman, R.A. Nelson, M. Prabhudesai, W.D. Savage, A.I. Nelson and L.W. Winter, 1993. Depression of plasma cholesterol in men by consumption of baked products containing soy protein. Am. J. Clin. Nutr., 58: 501-506.

Rimoldi, O.J., G.S. Finarelli and R.R. Brenner, 2001. Effectes of diabetes and insulin on hepatic Delta 6 desaturese gene expression. Biochem. Biophys. Res. Commun., 283 (2): 323-326.

Santiago, L.A., M. Hiramatsu and M. Mori, 1992. Japanese soybean pastemiso scavenges free radicals and ihhibits lipid peroxidation. J. Nutr. Sci. Vitaminol., 38: 297-304.

Sirtori, C.R., 2001. Risks and benefits of soy phytoestrogens in cardiovascular diseases, cancer, climacteric symptoms and osteoporosis. Drug Safety, 24: 665-682.

Sonee, M., T. Sum, C. Wang and S. Mukherjee, 2004. The Soy isoflavone, genistein, protects human cortical neuronal cell, from oxidative stres. Neuro. Toxicol., 25: 885-891.

Torres, N., I. Torre-Villalvazo and A.R. Tovar, 2006. Regulation of lipid metabolism by soy protein and its implication in diseases mediated by lipid disorders. J. Nutr. Biochemist., 17 (6): 365-373.

Tovar, A.R., I. Torre-Villalvazo, M. Ochoa, A.L. Elias, V. Ortiz and C.A. Aguilar-Salinas, 2005. Soy protein reduces hepatic lipotoxicity in hyperinsulinemic obese Zucker fa/fa rats. J. Lipid Res., 46: 1823-1832.

Towle, H.C., E.N. Kaytor and H.M. Shih, 1996. Metabolic regulation of hepatic gene expression. Biochem. Soc. Trans., 24: 364-368.

Umphress, S., S.P. Murphy, A.A. Franke, L.J. Custer and C.L. Blitz, 2005. Isoflavone content of foods with soy additives. J. Food Compos. Analy, 18 (6): 533-550.

Van Bilsen, M., J.E. deVries and G.J. VanderVusse, 1997. Long-term effects of fatty acids on cell viability and gene expression of neonatal cardiac myocytes. Prostaglandins, Leukotrienes and Essential Fatty Acids, 57 (1): 39-45.

Van der Vusse, GJ., J.F.C. Glatz, F.A. Van Nieuwenhoven, R.S. Reneman and J.B. Bassingthwaighte, 1998. Transport of long-chain fatty acids across the muscular endothelium. Skeletal Muscle Metabolism in Exercise and Diabetes Advances in Experimental. Med. Biol., 441: 181-191.

Wang, Z.R., J.G. Zou, Y.Z. Huang, K.J. Cao, Y.N. Xu and J.M. Wu, 2002. Effect of resveratrol on platelet aggregation *in vivo* and *in vitro*, Chinese. Med. J., 115: 378-380.