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◈ Research Note ◈

Anticarcinogenic Isoflavones in Soy Germ is Transferred to Egg Yolk

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Soy isoflavones have drawn much attention due to their potential to prevent breast and prostate cancers. This study examined whether the isoflavones present in soy germ, one of the richest natural sources of isoflavones, were transferred into eggs in laying hen or not. Eggs from laying hens fed diet containing 5 or 10% soy germ contained significantly higher amount of genistein and daidzein compared to eggs from laying hens fed control diet, suggesting the ability of isoflavones in soy germ to be digested, absorbed and accumulated in eggs. This finding raises the possibility to develop isoflavones-enriched eggs as a potential cancer preventive food.

Key Words: Soy germ, Isoflavones, Egg

INTRODUCTION

Dietary intake of soybean has been associated with low incidence of chronic diseases such as breast and prostate cancers, osteoporosis and cardiovascular disease.[1,2] Soybeans contain relatively high level of isoflavones, primarily in the various forms of beta-glycosides.[3] These conjugated isoflavones undergo hydrolysis by intestinal and /or microbial enzymes, releasing aglycones, genistein, daidzein and glycitein that are absorbed into intestine or further metabolized by microbial flora in large intestine.

Defatted soybean meals, which contain isoflavones, have widely been used in animal feeds. Isoflavones ingested as feeds are believed to be biologically transformed to their metabolites and sometimes accumulated in animals. Indeed, equol has been identified in the urine of some animals, indicating the metabolic conversion of isoflavone to a more active form of phytoestrogen.[4] Soybean germ is known be a rich source of isoflavones, with more than 70 umoles/g sample. We hypothesized that laying hen fed diet containing soy germ would produce eggs with varying amount of isoflavones according to period exposed to the diet. If successful, eggs enriched with bioactive isoflavones will be commercially available with low cost.

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MATERIALS AND METHODS

1) Preparation of isoflavone-enriched diet

Control diet was mainly prepared from yellow corn (54%), wheat bran (7%), corn gluten meal (3%), canola meal (1.5%), soybean meal (20.7%), oil (1.2%), stone powder (10.4%) and defluorinated calcium phosphate (1%), vitamin mixture (0.14%), and mineral mixture (0.13%). Experimental isoflavone-enriched diets were prepared simply by adding 5 or 10% soybean germ to control diet. Soybean germ contained moisture (7%), ash (4.0%), ether-extract (11.7%), crude protein (35.5%), and carbohydrate (41.8%). The composition of isoflavones in soy germ was as follows; total genistein 7 mg/g, total daidzein plus glycitein 35 mg/g.

2) Feeding trial

Fifteen laying hens (15-month old) were randomly assigned to receive control, 5% soy germ and 10% soygerm diets, with 5 hens in each treatment. Hens in all groups gave free access to diet and tap water. Average diet intake was estimated to be 105 g per day with no significant difference among experimental groups. Eggs were harvested daily from the first day of treatment for 21 days.

3) Isoflavone extraction from eggs

Egg white and yolk from each were mixed thoroughly by stirring prior to freeze-drying. Ten gram of egg powder was put into test tube with 20 ml 0.1 M acetate buffer (pH 5.0), followed by gentle vortex to make suspension. Egg suspension was mixed with 14,000 units beta-glucuronidase from *Helix pomatia* and incubated overnight at 37^{o}C. This step will release free isoflavones from conjugated isoflavones. Isoflavones was extracted by adding 3 volumes of ethanol and centrifuging at 2,000 g for 10 min. Supernatant was collected and 70% ethanol was added to the pellet, made suspension and centrifuged again. Supernatants from 2 extractions were pooled and mixed with a half volume of water. And fat-soluble substances in supernatant were removed by extracting with 15 ml hexane twice and isoflavones were extracted with diethyl ether (69, 20, and 15 ml) three times. After evaporating diethyl ether in vacuum rotary evaporator (Eyela, Tokyo, Japan), dried sample was resuspended in small amount of methanol and used for isoflavones analysis.

4) Analysis of isoflavones

Analysis of isoflavones was carried out using reversed-phase separation of the compounds on a C18 column (Shiseido, Japan) and using methanol /1 mM ammonium acetate (60/40) as isocratic eluent. A high-pressure liquid chromatography (Jasco PU 1580) equipped with UV/Visible detector (Jasco UV-2077, Japan). Elution was monitored at 254 nm (diet samples), and spectral data were recorded and stored over the time of the run on all samples.

RESULTS AND DISCUSSSION

Isoflavones is known to be concentrated in germ portion of soybean.[5] In soy processing such as soymilk preparation, germ is in most cases removed from the rest of it. Accordingly, soy germ may be used as relatively inexpensive source of isoflavones for foods and animal diet. Current study examined the potential of isoflavones in soy germ to be carried over to eggs of laying hen fed diet supplemented with soy germ.

As shown in Fig. 1 and 2, the level of isoflavones in eggs was significantly elevated in eggs from laying hens fed diet containing 10% soy germ from 1 week after feeding experimental diet. Diet supplemented with 5% soy germ showed marginal accumulation of isoflavones in eggs. Furthermore, the concentration of isoflavones in egg was gradually increased with feeding period experimental diet, with reaching maximum level (8 ug/g daidzein and 6 ug/g genistein) at 21st day (3 weeks) after feeding of experimental diet. Isoflavone contents in eggs from control appeared to increase during experimental period. This might be due

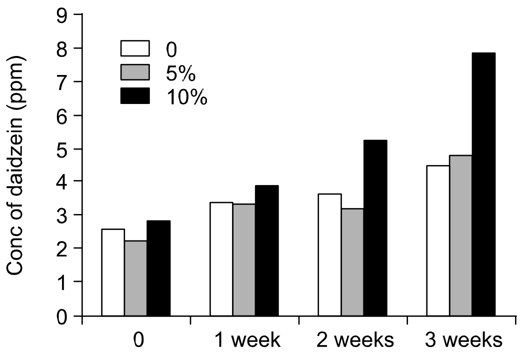

Fig. 1. Changes in the concentration of total daidzein in egg during the feeding of experimental diet. The laying hens were fed control diet (□), control diet supplemented with either 5% soy germ (▩) or 10% soy germ (■) for 3 weeks.

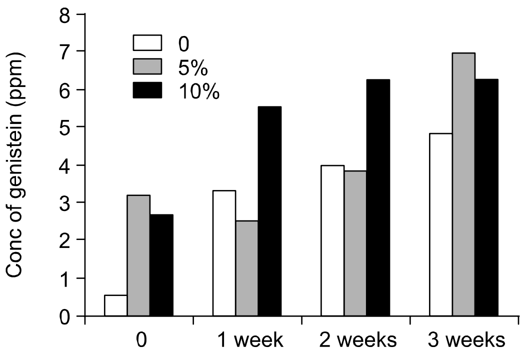

Fig. 2. Changes in the concentration of total genistein in egg during the feeding of experimental diet. The laying hens were fed control diet (□), control diet supplemented with either 5% soy germ (▩) or 10% soy germ (■) for 3 weeks.

to higher soy meal content in control diet than the conventional feed used until experiment began.

Saitoh and coworkers reported that isoflavones in eggs from laying hen fed diet containing 124 mg isoflavones per 100 g feed reached maximum level of 65.29 ug/100 g on the 12th day of the experiment, which is a significantly lower level compared to our study.[7)] The difference of isoflavone contents in eggs between Saitoh's and our study may be caused by the difference in dietary isoflavone concentration. We used diet supplemented with 5 or 10% soy germ which contains total 4,200 mg isoflavones per 100g feed which is 3 times higher than that used by Saitoh's group.

Egg is expected to contain glycitein as soy germ was known to have high concentration of glycitein.[5)] HPLC analysis system used for isoflavones analysis in the study did not resolve daidzein and glycitein clearly. Accordingly, the content of isoflavone designated as daidzein could in fact represent the sum of glycitein and daidzein.

Since isoflavones have binding affinity to estrogen receptor, it has been postulated that the compounds might interfere with hen's reproduction system and egg production. However, the weights of egg and egg yolk were ranged from 60～70 g and 17～19 g, respectively, and were not significantly different among treatment groups, suggesting that supplementation with the level of soy germ used in the study did not interfere with egg production.

Soybean isoflavones exist naturally in the glycoside form, and are hydrolyzed by intestinal glucosidases, which release the aglycones, daidzein, glycitein, and genistein.[8-10)] These are absorbed or further metabolized to various metabolites such as O-demethylangolensin and equol by intestinal microorganisms. The majority of aglycone forms of isoflavones absorbed would be changed into glucuronides and sulfide conjugate in the liver. Thereafter, the metabolites were transferred into blood, then peripheral tissue, and finally excreted in the feces and urine. One study reported that 74～90% daidzein and 91～96% of genistein existed as glucuronides in plasma from omnivorous and vegetarian women. Saitoh (2001) and coworkers reported that 70% of the isoflavones in egg yolk were present in conjugated form, making it easier for the isoflavones to be transferred into eggs.[7)]

Equol, a metabolite of daidzein, was also reported to be transferred to egg yolk although the compound was not detected in our study.[4,7)] The ability of equol to be transferred to egg proposes an important meaning because equol has much higher affinity to estrogen receptor than its parent compound. No particular attention has been paid to other foods than soy foods on the issue of isoflavones as dietary phytoestrogens. However, isoflavones from animal-based food might significantly contribute to health-promoting effect because their components have more estrogenic activity. It is also possible to develop the isoflavone-enriched eggs as heath foods. Further studies on how to increase isoflavones in egg and evaluate the effect of isoflavone-enriched animal foods on human health will be required.

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