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Safety evaluation of daidzein in laying hens: Part II. Effects on calcium-related metabolism

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ABSTRACT

Daidzein, an estrogen-like product, has become increasingly popular as a dietary supplement, particularly for postpeak-estrus animals seeking a safe natural alternative to play a role of estrogen. However, there is little available safety data of it for raisers and consumers. A subchronic laying hensafety study has been conducted to examine if the high-dose daidzein could affect calcium-related metabolism (eggshell quality and bone mineralization). Seven hundred and sixty-eight 56-week-old Hyline Brown were randomly assigned to 4 groups with 8 replicates of 24 birds each (192 laying hensper group) and 3 weeks later fed diets supplemented with 0(control), 10, 50 and 100 mg of daidzein/kg for 12 week. Eggshell thickness, eggshell percentage, eggshell strength, eggshell Ca concentration was increased linearly with increasing dietary daidzein supplementation ($P = 0.001$, $P = 0.007$, $P = 0.002$ and $P = 0.000$, respectively). Serum Ca increased linearly with increasing dietarydaidzein supplementation ($P = 0.042$), and serum P showed a significant quadratic response to dietarydaidzein supplementation ($P = 0.036$). Bone ash and bone Ca were significantly influenced by dietarydaidzein supplementation ($P < 0.05$). These findings indicate that daidzein hold no observed adverse effect on calcium metabolism, but also a safe and effective food additive for calcium metabolism in animals and humans.

1. Introduction

Soybeans are rich in isoflavones, which are naturally formed environmental estradiol synthesized from phenylpropanoids and simple phenols with a similar structure to estradiol. Epidemiological reports have associated soy products with preventing certain types of cancer, reducing the risk of osteoporosis, lowering plasma cholesterol, and acting as antioxidant agents and immune enhancers in humans and other animals (Messina, 2010). Two of the principle isoflavones in soybeans are genistein and daidzein (Lissin and Cooke, 2000). Soybeans contain approximately 670 mg genistein and 540 mg daidzein per kilogram (Franke et al., 1994). As the predominant isoflavone of soybeans, genistein has received more attention, reported to possess antioxidant and antiangiogenic properties and to inhibit protein tyrosine kinases (Martin et al., 2007). So more recent studies gradually began to investigate mechanisms of action and possible health benefits of daidzein (Liu and Zhang., 2008; Nynca et al., 2009; Atkinson et al., 2012).

It has been shown that the age of hens significantly decrease eggshell quality, especially shell strength (Rodriguez-Navarro et al., 2002). Recent studies have suggested that higher isoflavone intake is associated with increased bone mineral content, and reduced markers of bone resorption (Greendale et al., 2002; Fitzpatrick, 2003; Messina et al., 2004). Calcium plays an important role in the formation of eggshell and bone. Increasing calcium absorption might be responsible for increased eggshell strength or decreased occurrence of bone fracture. As age of birds advances demand for Ca may increase (Roland, 1979). Arjmandi et al. (2002), Fonseca and Ward (2004) showed that combination of daidzein and high Ca favorably affect cortical and trabecular bone as indicated by femur and lumbar vertebrae BMD and biomechanical strength.

In recent years daidzein has become increasingly popular as dietary supplements, particularly for postmenopausal women (Harris et al., 2002). While traditional corn and soybean-based poultry diets provide 7–10 mg of daidzein per day (Saitoh et al., 2001), commercially available purified supplements may deliver far greater daidzein amounts (>30 mg per serving)(Charles et al., 2006), despite little relevant safety data at these doses. Therefore, as part of an extensive programme of safety evaluation studies, we conducted a 84-day feeding study in laying hens to examine if high-dose daidzein could affect calcium-related metabolism (eggshell quality and bone mineralization). The high dose level was chosen to provide significant exposure to the test material and the other dose levels were set to provide data adequate for risk assessment purposes.

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Table 1
Ingredients and nutrient levels of experimental diet.[a]

Ingredients	Content (%)	Nutrient levels (analyzed)	
Corn	62.7	ME (Mcal/kg)	2.650
Soybean meal	26.3	CP (%)	16.61
Limestone	8.5	Ca (%)	3.5
DL-methionine	0.1	Available P (%)	0.35
Calcium hydrogen phosphate	1.0	Methionine (%)	0.35
50% Choline chloride	0.1	Lysine (%)	0.85
NaCl	0.3		
Vitamin and trace mineral[b] premix	1.0		

[a] Values are expressed on an air-dry basis.
[b] Premix provided per kilogram of diet: vitamin A (retinyl palmitate), 7715 IU; vitamin D3 (cholecalciferol), 2755 ICU; vitamin E (dl-α-tocopheryl acetate), 8.8 IU; vitamin K (menadione sodium bisulfate complex), 2.2 mg; vitamin B12(cobalamin), 0.01 mg; menadione (menadione sodium bisulfate complex), 0.18 mg; riboflavin, 4.41 mg; pantothenic acid (d-calcium pantothenate), 5.51 mg; niacin, 19.8 mg; folic acid, 0.28 mg; pyridoxine (pyridoxine hydrochloride), 0.55 mg; manganese (manganese sulfate), 50 mg; iron (ferrous sulfate), 25 mg; copper (copper sulfate), 2.5 mg; zinc (zinc sulfate), 50 mg; iodine (calcium iodate), 1.0 mg; selenium (sodium selenite), 0.15 mg.

2. Materials and methods

2.1. Birds, diet and management

This trial was carried out at the Poultry Institute, Chinese Academy of Agricultural Sciences. In this study, seven hundred and sixty-eight 56-week-old (the late stage of egg production cycle) Hyline Brown laying hens were randomly assigned to four groups with 8 replicates of 24 birds each (192 laying hens per group).

All birds were acclimated to the basal diet for 3 weeks. At 59 weeks of age, the birds were fed diets supplemented with 0 (control), 10 mg/kg (the maximum recommended available amount, MRAA), 50 mg/kg (five times MRAA) and 100 mg/kg (10 times MRAA) daidzein (Sichuan Guanghan Feed Co. Ltd., purity of 98.5%) for 12 week in the diets. Table 1 presents the composition of the experimental diets. Water and feed were provided ad libitum during the study. Four birds were housed in individual cages at dimensions of 50 × 50 cm, providing 625 cm^2 per bird. The photoperiod was set at 16L:8D throughout the study. Housing temperature and relative humidity were 20 ± 3 °C and 65–75%, respectively. All animal handling protocols were approved by The Poultry Institute Animal Care and Use Committee.

2.2. Sample collection and analytical determination

Sixty-four eggs per group (8 eggs/each replicate, 1 egg/each cage) were collected at the end of the study for measuring egg parameters. At 70 week of age, 8 reproductively active hens from each treatment group (n = 32) were killed by cervical dislocation. Blood samples were centrifuged at 3500*g* for 10 min and serum were collected. Left tibias were dissected out for subsequent analysis.

Egg quality parameters measured were eggshell thickness, eggshell weight, eggshell strength, eggshell Ca and P levels. Eggshell thickness was a mean value of measurements at three locations on the eggs (air cell, equator, and sharp end), measured with an Egg Shell Thickness Gauge (ESTG-1, ORKA Food Technology Ltd. Ramat Hasharon, Israel). Eggshell strength was measured with an Egg Force Reader (ORKA Food Technology Ltd. Ramat Hasharon, Israel). Serum Ca and P concentrations were measured using a biochemical analyser (UniCel DxC 800 Synchron, Beckman coulter, USA).

Tibias were frozen with flesh intact at −20 °C until further analysis. Before analysis, bones were cleaned of all tissue. The bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (Lunar Corp., Madison, WI, USA). Bone breaking strength was measured using an Instron universal testing machine with a 50-kg load cell at 50-kg load range with a crosshead speed of 50 mm/min with bone supported on a 3.00-cm span. Bone ash was determined on fat extracted, dried tibias. Bones were first oven-dried at 100 °C for 48 h, and then fat was extracted for 8 h using petroleum ether (boiling range 36–60 °C) with a Soxhlet extraction apparatus. Tibias were again oven-dried at 100 °C for 24 h, then ashed at 600 °C in a muffle furnace for 24 h for bone ash determination. Ca and P concentrations of eggshell and bone were analyzed by EDTA titration and colorimetric method respectively (AOAC, 2005).

2.3. Statistical analysis

All data were analyzed using SPSS (SPSS 16.0 for Windows, SPSS Inc., Chicago, IL, US). One-way ANOVA followed by a Duncan's multiple comparison test was used to separate different means among treatments. Data were assumed to be statistically significant when $p < 0.05$.

3. Results

3.1. Egg quality

The effect of dietary daidzein supplement on eggshell quality is shown in Table 2. Eggshell thickness, eggshell percentage, eggshell strength and eggshell Ca concentration were significantly influenced by dietary daidzein supplementation ($P < 0.05$), increasing linearly with increasing dietary daidzein supplement ($P = 0.001$, $P = 0.007$, $P = 0.002$ and $P = 0.000$, respectively). But there were no differences in eggshell P concentration ($P > 0.05$).

3.2. Serum Ca and P

The effect of dietary daidzein supplement on serum Ca and P was shown in Table 3. Dietary daidzein supplementation significantly affected serum Ca and P ($P < 0.05$). Serum Ca increased linearly with increasing dietary daidzein supplement ($P = 0.042$),

Table 2
Effect of dietary daidzein supplement on eggshell quality.[a]

Daidzein (mg/kg)	Eggshell thickness (mm)	Eggshell percentage (%)	Eggshell strength (kg/cm^2)	Eggshell Ca (%)	Eggshell P (%)
0	0.388	9.97	3.08	37.087	0.089
10	0.397	10.20	3.28	37.597	0.083
50	0.399	10.31	3.40	37.819	0.090
100	0.408	10.38	3.44	38.134	0.088
SEM	0.002	0.06	0.05	0.123	0.003
Probability					
Daidzein	0.006	0.047	0.013	0.004	0.867
Daidzein linear	0.001	0.007	0.002	0.000	0.865
Daidzein quadratic	0.955	0.455	0.300	0.604	0.736

[a] Results are means with n = 8 per treatment.

Table 3
Effect of dietary daidzein supplement on serum Ca and P.[a]

Daidzein (mg/kg)	0	10	50	100	SEM	Probability		
						Daidzein	Daidzein linear	Daidzein quadratic
Ca (mmol/L)	7.33	8.66	8.12	8.62	0.191	0.042	0.042	0.242
P (mmol/L)	2.56	3.18	3.03	2.96	0.084	0.043	0.115	0.036

[a] Results are means with n = 8 per treatment.