Effects of High-Dose Daidzein on Laying Performance, Egg Quality and Antioxidation in Laying Hens

Juan Cai¹, Huan Gu², Shourong Shi¹* and Haibing Tong¹*

¹ Poultry Institute, Chinese Academy of Agricultural Sciences, Yangzhou, Jiangsu 225125, P. R. China
² College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu Province 225009, P. R. China

This trial was conducted to evaluate the effects of high-dose daidzein supplements on laying performance, egg quality and antioxidation in laying hens during the late laying period. Seven hundred sixty eight 55-wk-old Hyline Brown were randomly assigned to four groups with 8 replicates of 24 birds each (192 laying hens per group) and fed diets supplemented with 0 (control), 10 mg/kg, 50 mg/kg and 100 mg/kg daidzein for 8 wk in the diets. Egg production, egg mass, feed conversion and egg yolk malondialdehyde were significantly effected by daidzein supplementation ($P<0.05$), while there are significant quadratic correlations between average egg production/egg mass/feed conversion/egg yolk malondialdehyde and daidzein supplementation (quadratic $P=0.022, 0.003, 0.021$ and $0.002$; respectively). The optimal daidzein supplementation in the diet of laying hens during the late laying period for maximum egg production, egg mass, feed conversion and egg yolk malondialdehyde were 72 mg/kg, 48.5 mg/kg and 56.34 mg/kg and 51.43 mg/kg. Daidzein supplementation significantly improved three calcium-related egg quality indexes (eggshell thickness, eggshell percentage and eggshell strength; $P<0.05$) and egg yolk total superoxide dismutase ($P<0.05$), linearly. These findings indicated that the daidzein could be a very effective additive to improve laying performance and eggshell quality in the birds during the late laying period, even though the dose of it was high.

Key words: antioxidation, daidzein, egg quality, laying hen, laying performance


Introduction

Soy products containing phytoestrogens have received much attention as dietary components to promote better health, such as preventing certain types of cancer, reducing the risk of osteoporosis, lowering plasma cholesterol, acting as antioxidant agents and immune enhancers in humans and animals (reviewed in Messina, 2010). The predominant isoflavone components of soybeans are genistein and daidzein. Soybeans contain approximately 670 mg genistein and 540 mg daidzein per kilogram (Franke et al., 1994). As the predominant isoflavone of soy, genistein has received more attention, reported to possess antioxidant and antiangiogenic properties and to inhibit protein tyrosine kinases (reviewed in Martín et al., 2007). So more recent studies gradually began to investigate mechanisms of action and possible health benefits of daidzein.

The physiologic effects of daidzein have been studied extensively in recent years (Messina, 2010), although public health recommendations regarding daidzein intake remain controversial. Much of this uncertainty has focused on potential adverse effects of daidzein in estrogen-sensitive tissues and metabolites. Due to the structural similarities to estrogens, daidzein could bind and transactivate estrogen receptors (Martin et al., 1978), induce proliferation in estrogen-sensitive endometrial and breast tumor cells in culture (Markiewicz et al., 1993; Wang et al., 1996; Hsieh et al., 1998), and elicit clear estrogenic effects in rodent models, particularly when given as purified supplements (Hsieh et al., 1998; Ju et al., 2001; Allred et al., 2004). Naturally, this evidence has raised concern that high levels of daidzein intake may promote estrogen-responsive negative effect in women and female animals.

In recent years daidzein has become increasingly popular as dietary supplements, particularly for postpeak-estrous animals seeking a safe natural alternative to traditional hormone therapies. 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), despite little relevant safety data at these doses. The purpose of this study was to examine if the high-dose daidzein could
affect the performance, egg quality and antioxidation in laying hens, providing some available safety information of daidzein.

Materials and Methods

1. Experimental Design

This trial was carried out at the Poultry Institute, Chinese Academy of Agricultural Sciences. In this study, seven hundred sixty eight 56-wk-old (the late stage of egg production cycle) Hyline Brown were randomly assigned to four groups with 8 replicates of 24 birds each (192 laying hens per group) and fed diets supplemented with 0 (control), 10 mg/kg, 50 mg/kg and 100 mg/kg daidzein (white powder, Sichuan Guanghan Feed Co. Ltd., purity of 98.5%) for 8 wk in the diets. Four birds were housed in individual cages at dimensions of 50 × 50 cm, providing 625 cm² per bird. Table 1 presents the composition of the experimental diets. Water and feed were provided ad libitum during the study. All birds were acclimated to the basal diet for 3 wk. The photoperiod was set at 16L:8D throughout the study. Housing temperature and relative humidity was 20 ± 3°C and 65 to 75 %, respectively. All animal handling protocols were approved by The Poultry Institute Animal Care and Use Committee.

2. Sample Collection and Analytical Determination

Body weights of laying hens were determined at the beginning and end of the study. Feed consumption was recorded on a replicate basis at weekly intervals. Daily egg production and egg weight were monitored during the trial. Egg production is expressed as average hen-day production, calculated from total eggs divided by the total number of hen-days. Feed conversion was expressed as g feed consumed /g egg produced. Eggs were examined for interior and exterior quality. 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.

Albumen height, Haugh unit and yolk colour were measured with an Egg Analyzer (ORKA Food Technology Ltd., Ramat Hasharon, Israel). The yolk colour is defined according to the Roche yolk colour fan, and colours should be within the range of 1-15 (1 represents bright yellow colour and 15 represents dark yellow colour). Eggshell strength was measured with an Egg Force Reader (ORKA Food Technology Ltd. Ramat Hasharon, Israel). Eggshell thickness was a mean value of measurements at 3 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). Egg-shape index was measured with a Digital Caliper (Guilin Guanglu Measuring Instrument Co., Ltd. Guangxi, P.R. China), and the shape index was calculated according to the formula: shape index = (height/width) × 100. After separating from albumen, the yolk was weighed and the mass of albumen was calculated as the difference in egg weight minus yolk and shell weight. The percentage was calculated as a ratio of egg weight.

The activities of superoxide dismutase (SOD) and the contents of malondialdehyde (MDA) were assayed using colorimetric methods with a spectrophotometer (722N, Shanghai Precision and Scientific Instrument Co. Ltd. Shanghai, P.R. China). The assays were conducted using the assay kits purchased from Nanjing Jiancheng Institute of Bioengineering (Nanjing, Jiangsu, P.R. China) and the procedures accordingly. All samples were measured in triplicate, at appropriate dilutions, to give activities of the enzymes in the linear range of standard curves constructed with pure enzymes. Protein content of supernatants was determined using the Coomassie Brilliant Blue G250 (Sigma Chemical, St. Louis, MO) assay with bovine serum albumin. The values are expressed as units per ml.
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 \( p<0.05 \). The replicate of laying hens was the experimental unit for all data.

Results

1. Laying Performance

Egg production, feed intake, egg mass, feed conversion, egg weight and mortality of laying hens in the four groups are shown in Table 2. Egg production, egg mass and feed conversion of whole experiment period were significantly influenced by dietary daidzein supplement \((P<0.05)\), showing significant quadratic response to increasing dietary daidzein supplement \((P=0.022, P=0.003 \text{ and } P=0.021, \text{ respectively})\), while feed intake and egg weight were not significantly effected \((P>0.05)\). The respective regression equations of quadratic response related to egg production, egg mass and feed conversion were: \(y=85.283+0.148x−0.001x^2\), \(y=54.856+0.097x−0.001x^2\), \(y=2.150−0.004x+0.000355x^2\). According to these three models, the optimal daidzein supplementations in the diet of laying hens for best egg production, egg mass and feed conversion were 72 mg/kg, 48.5 mg/kg and 56.34 mg/kg, respectively.

2. Egg Quality

Effect of daidzein on egg quality was shown in Table 3. Eggshell thickness, eggshell percentage and eggshell strength, all related with calcium, were significantly effected by dietary daidzein supplement \((P<0.05)\), showing significant linear response to increasing dietary daidzein supplement \((P=0.024, P=0.002 \text{ and } P=0.014, \text{ respectively})\), while there was no differences in egg shape index, egg yolk percentage, albumen percentage, albumen height, yolk color, Haugh Unit and eggshell color \((P>0.05)\).

3. Antioxidation

Effects of daidzein on SOD and MDA of egg yolk are shown in Figure 1 and 2. Egg yolk SOD and MDA were significantly effected by dietary daidzein supplement \((P<0.05)\). The egg yolk MDA production showed significant quadratic response to increasing dietary daidzein supplement \((P=0.002); y=241.515+2.160x−0.021x^2\). According to this model, the optimal daidzein supplementations in the diet of laying hens for maximum egg yolk MDA was 51.43 mg/kg.

Discussion

1. Laying Performance

The present study provided evidence that dietary daidzein significantly effected the egg production, egg mass and the feed efficiency during the postpeak period of egg laying in Hyline Brown hens (Table 2). It was confirmed by Zhao et
al. (2004, 2005) that daidzein supplementation improved postpeak egg production of Shaoxing ducks in a dose-dependent manner. Ni et al. (2007) and Liu and Zhang (2008) also found similar results in laying hens. This could be because daidzein serves as an agonist to occupy vacant estrogen-binding sites resulting in a total increase of the systemic estrogenic effect, upregulating mRNA expression of gonadotropin receptors to improve follicle development in chicken developing follicles and laying performance after the peak laying period (Cassidy, 2003; Liu and Zhang, 2008). Except for agonistic effect, daidzein can also act as an antagonist by competing with estrogen for estrogen receptors and producing a much weaker estrogenic response when endogenous estrogen is high (Setchell and Cassidy, 1999). This was ascertained by the present study that the egg production, egg mass, and feed efficiency of birds showed significant quadratic response to increasing dietary daidzein supplement. Further work of our study is needed to make some pathology analysis in ovary and oviduct.

2. Egg Quality

It is well known that calcium plays an important role in the formation of eggshell. Soy isoflavones have been shown to decrease intracellular calcium concentrations in osteoclasts (Gao and Yamaguchi, 1998; Kajiya et al., 2000), implying increased calcium amount for eggshell formation (De Matos, 2008). The improvement of three calcium-related egg quality indexes (eggshell thickness, eggshell percentage, and eggshell strength) in the present study was therefore probably due to increases in calcium absorption or retention. This result is in agreement with the results of the study by Ni et al. (2007) on daidzein in which daidzein supplement significantly increased the eggshell thickness, and decreased the percentage of cracked eggs. Sahin et al. (2007) also found that soy isoflavone supplementation could significantly improve bone mineral density, serum osteocalcin, vitamin D, calcium, phosphorus levels and alkaline phosphatase activity in quail during the late laying period.

3. Antioxidation

The extent of lipid peroxidation by reactive oxygen species can be monitored by MDA levels (Sumida et al., 1989). The concentrations of MDA in plasma and tissue are significantly decreased by ISF (soy isoflavones) treatment in male rabbits (Yousef et al., 2004) and broilers (Jiang et al., 2007). We could not compare our results with others because to our knowledge, there is no published previous study evaluating daidzein effect on antioxidative status of egg yolk (e.g., MDA, SOD, AOC) in laying hens. In the present study, MDA production of the egg yolk showed significant quadratic response to increasing dietary daidzein supplement. This finding suggested that the oxidation process of daidzein in the egg may be different from plasma and tissues in the laying hens.

Previous study (Jiang et al., 2007) reported that the addition of 40 or 80 mg/kg of soybean isoflavones to the diet slightly elevated SOD activity. It was consistent with present study that the activity of SOD in egg yolk significantly increased by adding 50 mg of daidzein/kg to diets, implicating that daidzein improved antioxidative status of laying hens by elevating the activity of antioxidant enzymes.

In conclusion, data obtained in the present study showed that suitable and available daidzein supplement could improve egg production, egg mass, feed efficiency, and egg yolk MDA, while excessive supplement could deteriorate them. It also enhanced the egg quality related with calcium metabolism. These findings indicated that the daidzein could be a very effective additive to improve laying performance and egg quality in the birds during the late laying period, even though the dose of it was high.

Acknowledgments

This work was financially supported by the Scientific and Technical Support Program of the “Twelfth Five-Year Plan”, Ministry of Science and Technology (2012BAD39B04); Special Fund for Agro-scientific Research in the Public Interest of Ministry of Agriculture (201003011); National Spark Program Project (S2011C100476), Ministry of Science and Technology; Independent Innovation Program of Agriculture Science and Technology, Jiangsu Province (CX(11)
References


