

## Effects of Soybean Isoflavone on Production Performance, Egg Quality, and Plasma Hormone Indices in Local Laying Hens during the Late Laying Period: Postprint

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### Abstract

This experiment aimed to investigate the effects of dietary daidzein supplementation on production performance, egg quality, and plasma hormone indices in local laying hens during the late laying period. A total of 192 55-week-old Rugao Yellow laying hens with similar body weight and laying rate were randomly allocated into 2 groups, with 6 replicates per group and 16 hens per replicate. The control group was fed the basal diet, while the experimental group was fed the basal diet supplemented with 60 mg/kg daidzein. The experiment consisted of a 2-week preliminary period followed by a 14-week formal experimental period. The results showed that: 1) The laying rate of the experimental group was significantly higher than that of the control group ( $P < 0.05$ ), while the mortality rate, average egg weight, average daily feed intake, and feed-to-egg ratio did not differ significantly between the two groups ( $P > 0.05$ ). 2) The Haugh unit of eggs in the experimental group was significantly higher than that in the control group ( $P < 0.05$ ), while other egg quality indices did not differ significantly between the two groups ( $P > 0.05$ ). 3) The plasma estradiol (E2) content in the experimental group was significantly higher than that in the control group ( $P < 0.05$ ), while the plasma follicle-stimulating hormone (FSH) content was significantly lower ( $P < 0.05$ ); other plasma hormone indices did not differ significantly between the two groups ( $P > 0.05$ ). In conclusion, dietary supplementation with 60 mg/kg daidzein can improve production performance, egg quality, and plasma hormone indices in local laying hens during the late laying period.

## Full Text

# Effects of Daidzein on Performance, Egg Quality and Plasma Hormone Indices of Local Laying Hens during Late Laying Cycle

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**Abstract:** This experiment investigated the effects of dietary daidzein supplementation on performance, egg quality, and plasma hormone indices in local laying hens during the late laying cycle. A total of 192 fifty-five-week-old Rugao yellow-feathered laying hens with similar body weight and laying rate were randomly assigned to two groups, each consisting of six replicates of sixteen hens. The control group received a basal diet, while the experimental group received the basal diet supplemented with 60 mg/kg daidzein. The study included a 2-week pre-trial period followed by a 14-week formal experimental period. The results demonstrated that: (1) the laying rate of the experimental group was significantly higher than that of the control group ( $P < 0.05$ ), whereas mortality, average egg weight, average daily feed intake, and feed-to-egg ratio showed no significant differences ( $P > 0.05$ ); (2) the Haugh unit of eggs from the experimental group was significantly higher than that of the control group ( $P < 0.05$ ), while other egg quality parameters showed no significant differences ( $P > 0.05$ ); and (3) plasma estradiol (E2) content in the experimental group was significantly higher ( $P < 0.05$ ), while plasma follicle-stimulating hormone (FSH) content was significantly lower ( $P < 0.05$ ) compared to the control group, with no significant differences observed in other plasma hormone indices ( $P > 0.05$ ). In conclusion, dietary supplementation with 60 mg/kg daidzein can improve the performance, egg quality, and plasma hormone profiles of local laying hens during the late laying cycle.

**Keywords:** daidzein; laying hens; performance; egg quality; hormone

Daidzein, a naturally occurring compound found in legumes, red clover, and kudzu, is classified as a phytoestrogen due to its structural similarity to estradiol (E2) and its ability to bind estrogen receptors (ERs). Known for its beneficial effects in alleviating menopausal symptoms, reducing cancer risk, and providing antioxidant activity in humans, daidzein has attracted increasing attention as a feed additive in animal production. As laying hens enter the late laying cycle, ovarian function begins to decline, accompanied by decreased estrogen levels, which not only reduces laying rate but also compromises egg quality, leading to an increased incidence of shell defects. Therefore, investigating the effects of daidzein on late-cycle laying hens holds significant practical importance for extending the productive lifespan and improving economic efficiency of layer flocks. Previous studies across various poultry species have demonstrated that soy isoflavones can significantly improve production performance. Research from

our group and others has shown that dietary daidzein supplementation at appropriate levels can enhance laying performance in late-cycle Hy-Line Brown hens and breeding chickens. However, limited information is available regarding its application in local chicken breeds. This study therefore selected Rugao yellow-feathered laying hens as a model to evaluate the effects of dietary daidzein on production performance, egg quality, and plasma hormone indices, aiming to provide a reliable basis for improved management of local chicken breeds.

### **1.1 Experimental Materials and Animals**

The experimental material was daidzein (4,7-dihydroxyisoflavone >97.5%), a white powder supplied by Sichuan Guanghan Biochemical Products Co., Ltd. The experimental animals were Rugao yellow-feathered laying hens.

### **1.2 Experimental Design**

One hundred ninety-two fifty-five-week-old Rugao yellow-feathered laying hens with similar body weight and laying rate were randomly allocated into two groups, each comprising six replicates of sixteen hens. The control group was fed a basal diet, while the experimental group received the basal diet supplemented with 60 mg/kg daidzein (based on our laboratory's previous findings in Hy-Line Brown hens, this dosage was selected). The composition and nutrient levels of the basal diet are presented in Table 1. The experiment consisted of a 2-week pre-trial period and a 14-week formal trial period.

### **1.3 Management Practices**

Hens were housed in tiered cages and fed twice daily at 08:00 and 15:00 with ad libitum access to feed and water. Conventional management practices were applied identically to both groups.

#### **1.4.1 Production Performance**

Eggs were collected daily at 15:00, and the number of eggs, defective eggs, egg weight, and mortality were recorded accurately by replicate. Feed consumption was measured and feed was weighed at the end of each week. Based on these records, mortality rate, laying rate, average daily feed intake, average egg weight, and feed-to-egg ratio were calculated.

#### **1.4.2 Egg Quality**

At week 16 of the experimental period, three eggs were randomly selected from each replicate (eighteen eggs per group) for egg quality analysis. Parameters measured included egg shape index, shell thickness, yolk ratio, albumen ratio, shell ratio, shell strength, albumen height, and Haugh unit. The primary instruments used were an eggshell thickness gauge, eggshell strength tester (ORKA,

Israel), CM-2300d spectrophotometer (MINOLTA, Japan), and electronic digital calipers (Guanglu Digital Measurement and Control Co., Ltd.).

#### **1.4.3 Plasma Hormone Indices**

At the end of the experiment, two hens from each replicate were selected for blood collection (3 mL from the wing vein) into anticoagulant tubes. Plasma was harvested after centrifugation at 3,000 r/min for 10 minutes. Radioimmunoassay (RIA) was employed to determine plasma concentrations of E2, progesterone (P4), follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroxine (T4), and triiodothyronine (T3). All assay kits were provided by Beijing North Institute of Biotechnology.

#### **1.5 Statistical Analysis**

Data were processed using SPSS 21.0 statistical software. Inter-group differences were analyzed using t-tests, with  $P < 0.05$  considered statistically significant. Results are expressed as mean  $\pm$  standard error (mean  $\pm$  SE).

#### **2.1 Effects of Daidzein on Mortality and Production Performance of Late-Cycle Laying Hens**

The effects of daidzein on mortality and production performance are shown in Table 2. Daidzein supplementation significantly affected laying rate ( $P < 0.05$ ), with the experimental group showing a 3.4% improvement compared to the control group ( $P < 0.05$ ). No significant effects were observed on average daily feed intake, average egg weight, feed-to-egg ratio, or mortality rate ( $P > 0.05$ ).

#### **2.2 Effects of Daidzein on Egg Quality of Late-Cycle Laying Hens**

The effects of daidzein on egg quality are presented in Table 3. Daidzein significantly influenced Haugh unit ( $P < 0.05$ ), with the experimental group exhibiting higher values than the control group ( $P < 0.05$ ). No significant effects were detected on other egg quality parameters ( $P > 0.05$ ).

#### **2.3 Effects of Daidzein on Plasma Hormone Indices of Late-Cycle Laying Hens**

The effects of daidzein on plasma hormone indices are summarized in Table 4. Daidzein significantly impacted plasma E2 and FSH concentrations ( $P < 0.05$ ). The experimental group showed significantly elevated E2 levels and significantly reduced FSH levels compared to the control group ( $P < 0.05$ ). No significant differences were observed in other plasma hormone indices ( $P > 0.05$ ).

### 3.1 Effects of Daidzein on Production Performance of Late-Cycle Laying Hens

As a natural phytoestrogen, daidzein exhibits structural similarity to 17- $\beta$ -E<sub>2</sub>, conferring both estrogenic activity and antioxidant capacity. Numerous studies have demonstrated that appropriate daidzein supplementation during the late laying cycle can significantly improve poultry reproductive performance. Ni et al. reported that dietary supplementation with 10 mg/kg daidzein significantly increased laying rate in 45-day-old Isa Brown hens. Zhao et al. observed improved reproductive performance in Zhedong white geese fed diets containing 10 or 20 mg/kg daidzein. Zhao Fengqi and colleagues found that feeding 44-week-old Hy-Line Brown hens diets supplemented with 10 or 15 mg/kg daidzein for 12 weeks significantly enhanced laying rate. Gu Huan et al. demonstrated that 10 mg/kg daidzein increased egg weight, while 50 mg/kg daidzein improved laying rate and reduced feed-to-egg ratio without affecting average daily feed intake in late-cycle laying hens. Our findings align with those of Gu Huan et al., showing that 60 mg/kg daidzein significantly improved laying rate in late-cycle hens. This effect may be attributed to daidzein's estrogenic action, which ameliorates estrogen deficiency and promotes follicular development during the late laying period. Some studies have reported significant improvements with lower daidzein doses (10-20 mg/kg), and these discrepancies may relate to differences in breed sensitivity, age at supplementation, and other factors requiring further investigation.

### 3.2 Effects of Daidzein on Egg Quality of Late-Cycle Laying Hens

Avian egg production is a complex physiological process involving ovarian development, yolk deposition, hierarchical follicle formation, and shell formation. Shell quality is regulated by multiple factors, and several studies have shown that 10 mg/kg daidzein supplementation in late-cycle broiler breeders significantly increased shell thickness, specific gravity, and strength. The proposed mechanism involves daidzein's ability to reduce calcium ion concentration in osteoclasts while enhancing calcium absorption, ultimately increasing shell calcium content. In contrast, our supplementation with 60 mg/kg daidzein did not significantly affect shell thickness or strength, possibly due to differences in hen age or supplementation duration, though the exact reasons remain unclear. The Haugh unit serves as a crucial indicator of egg quality, with higher values indicating better albumen viscosity and quality. Previous research demonstrated that 10 mg/kg daidzein significantly improved Haugh unit and egg freshness in late-cycle broiler breeders. Our study similarly found that 60 mg/kg daidzein significantly elevated Haugh unit, indicating improved albumen quality. Therefore, appropriate daidzein supplementation can enhance egg freshness and overall quality.

### 3.3 Effects of Daidzein on Plasma Hormone Indices of Late-Cycle Laying Hens

Hormonal regulation through the neuroendocrine system plays a critical role in avian reproduction. Studies have shown that daidzein can bind to ERs in the hypothalamus and pituitary, thereby improving reproductive function. Estrogen is pivotal in the reproductive axis, and research using 50-week-old Nongda-3 dwarf hens demonstrated that 10 mg/kg daidzein significantly increased plasma E2 levels. Similarly, studies in 70-week-old Hy-Line Brown hens showed that 15 mg/kg daidzein significantly promoted E2 secretion. Zhao et al. reported that daidzein elevated plasma E2 and T4 levels in 402-day-old Shaoxing ducks, concurrent with increased laying rate, suggesting estrogen involvement in daidzein's mechanism of action. Our results are consistent with these findings, showing that 60 mg/kg daidzein increased plasma E2 concentration by approximately 70%. FSH, a protein hormone secreted by the pituitary, promotes follicular development. Research in menopausal women has shown that decreased plasma E2 is accompanied by elevated FSH levels. Studies in aging female rats demonstrated that daidzein supplementation increased plasma E2 while decreasing FSH. Our findings align with these results, with the experimental group showing significantly lower plasma FSH levels. We hypothesize that late-cycle laying hens experience a physiological state similar to menopausal women or aging female rats, and that daidzein supplementation restores estrogen levels, reduces excessive FSH, and rebalances the hypothalamic-pituitary-ovarian axis, thereby alleviating ovarian senescence and prolonging peak production.

### Conclusion

Under the conditions of this experiment, dietary supplementation with 60 mg/kg daidzein improved production performance and egg quality while modulating endocrine hormone levels in local laying hens during the late laying cycle.

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