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# EFFECTS OF DIETARY SUPPLEMENTATION WITH ISOFLAVONES ON EXTERIOR DEVELOPMENT AND TIBIA BONE QUALITY OF LAYING HENS

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## ABSTRACT

The experiment was conducted to evaluate the effect of dietary supplementation with isoflavones on exterior development and tibia bone quality of laying hens. Eighty laying hens, 20 weeks old, were divided into 5 groups, 20 in each and fed with different amount of 300, 600, 1200 and 1800 mg.kg$^{-1}$ supplemented isoflavones (SI) in the feed. Dietary isoflavones supplemented in different concentrations to the ISA Brown laying hens diet caused increase of the body weight ($P < 0.05$). The heaviest body weight was noticed in the group fed with supplementation of 1800 mg.kg$^{-1}$ SI (2142.5 ± 190.5 g). In addition, shank length as an important measurement of skeletal development was significantly longest in the group fed with supplementation of 1800 mg.kg$^{-1}$ SI (29.50 ± 1.73 cm). There were no differences in other exterior parameters among the experimental groups ($P > 0.05$). High SI treatment (1800 mg.kg$^{-1}$ feed) had a beneficial effect ($P < 0.05$) on bone quality of aged laying hens (weight, volume, ash/tibia and calcium), but do not affect on the tibial phosphorus ($P > 0.05$). Results of the present study indicates that isoflavones are effective supplements to improve the body weight and calcium bone content during the late laying period, even though their supplementation was high.

**Key words:** isoflavones; genistein; daidzein; bone quality; laying hens

## INTRODUCTION

Commercial laying hens have a problem with the loss of bone strength during their egg production cycle. When the hens reached sexual maturity, the osteoporosis initiated serious welfare problems (Webster, 2004; Whitehead and Fleming, 2000). In their ordinary diet the soybean and its products are commonly used as a protein source for layers. Soybean is a great source of isoflavones, such as genistein and daidzein, which are believed to be biologically active in several animals, including humans and poultry. Supplementation of daidzein improves the laying performance during post peak laying in Shaoxing duck (Zhao *et al.*, 2005) and the amount of cracked eggs decreases and eggshell thickness and egg production increases (Ni *et al.*, 2007). Sahin N. *et al.* (2006) reported that soybean isoflavones have a significant effect on the egg quality and bone mineralization in quails.

The experiment will thus provide knowledge on the effect of dietary supplementation with isoflavones on the tibia bone quality in aging laying hens and may provide as a means to increase the welfare of laying hens.

## MATERIAL AND METHODS

### Animal Treatments and Doses

The experiment was performed on ISA Brown laying hens in a commercial poultry farm. Eighty laying hens, 20 weeks old, were divided into 5 groups,

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20 in each (4 cages for a group), set to a 16L:8D cycle and fed with different amount of supplemented isoflavones (SI) in the feed. The control group was blank and fed with no SI in the feed and the other 4 experimental groups was fed with SI in the feed in amount of 300, 600, 1200 and 1800 mg.kg$^{-1}$. Water was offered for *ad libitum* consumption throughout the experiment, which was conducted for 40 weeks. The experimental feed was supplemented with a concentrated product, 40 % isoflavones, produced by the North China Pharmaceutical Corporation. The isoflavone composition of the product is presented in Table 1.

The composition and nutritive value of the basal diet is presented in Table 2.

The laying hens received additional isoflavones from 20 to 60 weeks of age.

**Table 1: Composition of the 40 % isoflavone product**

| Isoflavone | % |
|---|---|
| 1. Genistin | 7.30 |
| 2. Genistein | 1.26 |
| 3. Daidzin | 22.12 |
| 4. Daidzein | 1.74 |
| 5. Glycitin | 8.01 |
| 6. Glycitein | 0.45 |
| Total | 40.88 |

**Exterior Parameters**

The body weight of the laying hens was measured with a special digital balance at the beginning and at the end of the experiment. The body length, the breast circumference and the length of the legs were measured using the clot tape.

**Table 2: Composition and nutritive value of the basal feed (BF)**

| Ingredient, g.kg$^{-1}$ | Basal feed (BF) Phase I of egg production | Basal feed (BF) Phase II of egg production | Basal feed (BF) Phase III of egg production |
|---|---|---|---|
| Yellow corn | 40.01 | 40.24 | 43.13 |
| Soybean meal, 44 % protein | 18.96 | 15.60 | 14.43 |
| Sunflower meal, 33 % protein | 15.00 | 15.00 | 15.30 |
| Wheat middilings | 9.40 | 12.39 | 10.70 |
| Soybean oil | 5.10 | 4.94 | 4.32 |
| Methionine, 99 % | 0.12 | 0.07 | 0.04 |
| Calcium carbonate | 9.12 | 9.61 | 9.94 |
| Monocalcium phosphate | 1.10 | 0.90 | 0.76 |
| $NaHCO_3$ | 0.15 | 0.20 | 0.30 |
| Potasium carbonate | - | 0.04 | 0.09 |
| Zeolites | 0.30 | 0.30 | 0.30 |
| Salt | 0.24 | 0.22 | 0.19 |
| Vitamin and mineral mixture | 0.50 | 0.50 | 0.50 |
| Total | 100.00 | 100.00 | 100.00 |
| Chemical composition, calculated | | | |
| Dry matter, g.kg$^{-1}$ | 904.30 | 904.00 | 903.10 |
| Metabolic energy, kcal.kg$^{-1}$ | 2800.00 | 2750.00 | 2750.00 |
| Crude proteins, g.kg$^{-1}$ | 179.90 | 169.40 | 165.00 |
| Crude fat, g.kg$^{-1}$ | 71.50 | 70.90 | 65.20 |
| Crude fiber, g.kg$^{-1}$ | 69.80 | 72.00 | 70.00 |
| Total ash, g.kg$^{-1}$ | 125.30 | 130.00 | 130.00 |
| Calcium, g.kg$^{-1}$ | 37.50 | 39.00 | 40.00 |
| Phosphorus (available), g.kg$^{-1}$ | 3.80 | 3.40 | 3.00 |
| Lysine, g.kg$^{-1}$ | 8.50 | 7.70 | 7.40 |
| DL Methionine, g.kg$^{-1}$ | 4.60 | 3.90 | 3.60 |
| Methionine + cystine, g.kg$^{-1}$ | 7.30 | 6.50 | 6.10 |

**Bone Sample Collection**

At the end of the experiment 5 laying hens from each experimental group were sacrificed. The laying hens were anaesthetized by inhalation with diethyl ether and killed. At necroscopy, the right tibia was excised from the body and defleshed without boiling. The tibia was then sealed in a plastic bag to minimize moisture loss and stored at -20 °C until analysis. The bone volume was taken by the weight change in water method. Tibias were measured on the air and in the water. The weight change equalled the weight of water replaced by the bone as the specific gravity of water is 1.0 $g.cm^{-3}$. The weight was measured with a digital balance of 0.01 g accuracy. Bone length was measured with a calliper with 0.01 cm accuracy. The tibia was defatted, dried at 105 °C for 24 h and placed in a dessicator. The bone ash weight was obtained after ashing at 600 °C for 24 h. Bone ash concentration was calculated as bone ash weight per unit of volume. The content of calcium and phosphorus were determined by potassium per manganate titration and vanadate-molybdate colorimetric methods, respectively.

**Statistical analysis**

Statistical analysis was performed by Statgraph 3 software package. One-way analysis of variance (ANOVA) was used for the differences between groups. When the F values were significant, the Duncan's Multiple Range Test was performed.

## RESULTS AND DISCUSSION

The obtained results of the body weight and the exterior parameters are presented in Table 3.

There are some information (Payne *et al*., 2001; Jiang *et al*., 2007) which indicate that phytohormones added to a large amount to the feed of chicken cause changes in the body weight and appearance that have influence on some other exterior characteristics and the whole body conformation. In our experiment dietary isoflavones supplemented in different concentrations to the ISA Brown laying hens' diet caused increase in the body weight ($P < 0.05$). The heaviest body weight was noticed in the group fed with supplementation of 1800 $mg.kg^{-1}$ SI (2142.5 ± 190.5 g).

In addition, shank length is an important measurement of skeletal development (Yilmaz Dickmen and Ipek, 2006). It is related with the productive traits of the laying hens (Petek *et al*., 2000). The longest shank length was noticed in the group fed with supplementation of 1800 $mg.kg^{-1}$ SI (29.50 ± 1.73 cm). This result is in agreement with the results of our previous study conducted with ISA Brown pullets (Gjorgovska *et al*., 2014).

**Table 3: Body weight and exterior parameters of the ISA brown laying hens**

| | Basal feed (BF) | Basal feed (BF) + 300 $mg.kg^{-1}$ SI | Basal feed (BF) + 600 $mg.kg^{-1}$ SI | Basal feed (BF) + 1200 $mg.kg^{-1}$ SI | Basal feed (BF) + 1800 $mg.kg^{-1}$ SI |
|---|---|---|---|---|---|
| Body weight, g | 1987.5 ± 125.8$^{b}$ | 1967.5 ± 100.1$^{bc}$ | 2045.0 ± 92.9$^{b}$ | 1955.0 ± 92.6$^{bc}$ | 2142.5 ± 190.5$^{a}$ |
| Body lenght, cm | 28.88 ± 1.55$^{ab}$ | 27.13 ± 0.85$^{b}$ | 28.50 ± 1.29$^{ab}$ | 27.50 ± 1.29$^{ab}$ | 29.50 ± 1.73$^{a}$ |
| Breast circumference, cm | 21.50 ± 1.73 | 20.63 ± 1.25 | 20.75 ± 0.65 | 20.75 ± 1.26 | 22.50 ± 1.29 |
| Length of femur, cm | 11.25 ± 0.96 | 11.00 ± 0.05 | 11.00 ± 0.82 | 10.75 ± 0.50 | 11.00 ± 0.71 |
| Length of tibia, cm | 20.50 ± 1.29 | 20.38 ± 0.95 | 22.50 ± 0.58 | 21.50 ± 0.58 | 21.38 ± 1.80 |
| Length of metatarsus, cm | 29.50 ± 1.29 | 29.00 ± 1.41 | 29.75 ± 0.50 | 29.50 ± 0.58 | 29.75 ± 1.50 |

SI – supplemented isoflavones; a,b,c – values in the same row with no common superscript differ significantly ($P < 0.05$).

There were no differences in other exterior parameters among the experimental groups ($P>0.05$).

There are few studies investigating the effects of the soy phytoestrogens on bone strength in animals, but this is one of the newest studies to investigate the effect of genistein and daidzein on the bone quality of laying hens during late egg production.

These results suggest that the high supplemented isoflavones treatment (1800 $mg.kg^{-1}$ feed) has a beneficial effect ($P < 0.05$) on bone quality of aged laying hens (weight, volume, ash/tibia and calcium), but do not affect on the tibial phosphorus ($P > 0.05$). Sahin *et al*. (2007) reported that supplementation with soy isoflavones significantly improve bone mineral density in Japanese

**Table 4: Bone quality and mineralization of the tibia (calculated on dry matter)**

| | Basal feed (BF) | Basal feed (BF) + 300 mg.kg$^{-1}$ SI | Basal feed (BF) + 600 mg.kg$^{-1}$ SI | Basal feed (BF) + 1200 mg.kg$^{-1}$ SI | Basal feed (BF) + 1800 mg.kg$^{-1}$ SI |
|---|---|---|---|---|---|
| 1. Weight of the tibia, g | $7.08 \pm 0.56^{ab}$ | $6.71 \pm 1.24^{b}$ | $7.45 \pm 0.77^{ab}$ | $7.06 \pm 0.77^{ab}$ | $8.38 \pm 1.01^{a}$ |
| 2. Volume of the tibia, cm$^3$ | $9.75 \pm 0.50^{b}$ | $8.38 \pm 0.75^{a}$ | $9.63 \pm 0.75^{b}$ | $9.83 \pm 0.29^{b}$ | $10.50 \pm 0.58^{b}$ |
| 3. Ash/ tibia, g | $4.10 \pm 0.37^{ab}$ | $4.12 \pm 0.62^{ab}$ | $4.18 \pm 0.53^{ab}$ | $3.89 \pm 0.56^{b}$ | $4.88 \pm 0.74^{a}$ |
| 4. Calcium, g | $2.68 \pm 0.17^{b}$ | $2.53 \pm 0.47^{b}$ | $2.97 \pm 0.27^{ab}$ | $2.61 \pm 0.21^{b}$ | $3.31 \pm 0.50^{a}$ |
| 5. Phosphorus, g | $1.03 \pm 0.32$ | $1.16 \pm 0.20$ | $1.21 \pm 0.42$ | $1.22 \pm 0.17$ | $1.39 \pm 0.15$ |

SI – supplemented isoflavones; Ash concentration1 (g.cm$^{-3}$) (Ash concentration = ash weight/bone volume); a, b – values in the same row with no common superscript differ significantly ($P < 0.05$).

quail. Recent studies also suggested that higher isoflavone intake is associated with increased mineral content (Saiafzadeh and Jahanian, 2013).

## CONCLUSSIONS

The results of the present study show that supplementation with 600 and 1800 mg isoflavones/kg diet increased the body weight significantly ($P < 0.05$). The longest shank length was noticed in the group fed with supplementation of 1800 mg.kg$^{-1}$ SI ($P < 0.05$). These findings also suggest that isoflavones have a stimulatory effect on calcium content in bone in groups supplemented with 1800 mg isoflavones .kg$^{-1}$ diet ($P < 0.05$). These results indicate that isoflavones are effective supplements to improve the body weight, body length and calcium bone content during the late laying period, even though their supplementation is high.

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