Effects of Gushukang, a Chinese herbal medicine, on bone characteristics and osteoporosis in laying hens


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ABSTRACT In this study, we evaluated the effects of the herb medicine formula Gushukang (GSK) on bone characteristics and osteoporosis in end-of-lay hens. One thousand 55-wk-old ISA caged layers were allotted randomly to 2 groups. The control group was given the basal diet, and the GSK group was given the basal diet supplemented with additional GSK (1 g/kg) for 10 wk. Egg production, shell quality, bone radiographic density, and biochemical markers of bone turnover were determined. The results showed that GSK significantly increased the egg laying rate and decreased the percentage of cracked eggs ($P < 0.05$). The serum calcium, phosphate, and alkaline phosphatase were decreased ($P < 0.05$) in the GSK-treated group compared with the control group, whereas bone characteristics were significantly improved ($P < 0.05$). The results suggested that GSK can improve egg production and prevent bone loss by inhibiting bone turnover.

Key words: Gushukang, egg laying production, bone characteristic, ISA cage layer

INTRODUCTION

Osteoporosis is a widespread cause of skeletal problems in laying hens. The loss of structural bone associated with this condition can increase the fragility of bones and contribute to high fracture incidence (Randall and Duff, 1988). Surveys of laying hens in the United Kingdom and European flocks have indicated that around 30% of the birds experience one or more bone fractures during their life. The high fracture incidences show that osteoporosis constitutes a severe health problem in hens. Production losses and mortality also arise with osteoporosis (Gregory and Wilkins, 1989). Some of the factors that lead to osteoporosis have been investigated. Exercise, husbandry system, nutrition, and genetics can all contribute to osteoporosis (Fleming et al., 1994; Rennie et al., 1997; Bishop et al., 2000). Structural bone formation during rearing occurs by normal adaptive remodeling. However, at the onset of sexual maturity, the function of osteoblasts changes from forming structural bone to medullary bone. The amount of medullary bone builds up rapidly and can continue to accumulate slowly during the laying period. In the absence of structural bone formation, osteoclastic resorption continues and the structural bone content of the hen declines, ultimately leading to osteoporosis (Whitehead and Fleming, 2000; Webster, 2004).

As in postmenopausal osteoporosis in women, the severity of the avian condition is likely to be dependent upon peak structural bone mass and the rate of subsequent structural bone loss. Some drugs for the treatment of postmenopausal osteoporosis have been used to alleviate osteoporosis in laying hens. The bisphosphonate alendronate appeared to stimulate the formation of cancellous bone and therefore prevented the bone loss with medullary bone modeling but not that which occurs during remodeling (Wilson et al., 1998). Fluoride has also been shown to result in a higher medullary bone content in hens at the end of the laying cycle, but increases in medullary content may nonetheless have a beneficial effect on bone quality (Fleming et al., 2003). These drugs are unlikely to be a practical solution for laying hen osteoporosis. In China, traditional Chinese medicines have been commonly used to treat postmenopausal osteoporosis in women with satisfactory results (Song et al., 2000; Wang et al., 2006; Shi et al., 2008). An herbal medicine formula, Gushukang (GSK), consists of 4 traditional Chinese drugs: *Herbal Epimedii*, *Rhizoma Drynariae*, *Rhizoma Atractylodis*, and *Radix Astragali*. *Herbal Epimedii* is derived from the leaves of the plant. *Rhizoma Drynariae*, *Rhizoma Atractylodis*, and *Radix Astragali* are all derived from the roots of the plants. Data from a previous study have shown that GSK can improve bone mineral content in young laying hens (Deng and Hou, 2003). The main objective of the present study was to determine the effects of GSK...
on bone characteristics and osteoporosis in end-of-lay hens.

MATERIALS AND METHODS

Materials

Gushukang was produced by Nanjing Animal Health Products Co. Ltd (Nanjing, China). Gushukang and its preparations were standardized and quality controlled according to the guidelines defined by the Ministry of Agriculture of the People’s Republic of China. Test kits for serum calcium, phosphate, and alkaline phosphatase (ALP) were purchased from Nanjing Jiancheng Biotechnology Ltd. (Nanjing, China). All other chemicals and reagents were of the highest analytical grade.

Birds, Husbandry, and Treatments

One thousand ISA laying hens were selected during the postpeak period of egg laying (55 wk of age, 70 to 85% egg laying rate), randomly allocated to control and GSK-treated groups (500 hens per group), and raised in 3-tier caged layer houses (2 hens per cage). These birds were diagnosed as having osteoporosis before experiment with the methods by Whitehead and Wilson (1992). The control group was fed with the basal diet, and the GSK group was fed the basal diet supplemented with GSK (1 g/kg) for 10 wk. The hens were fed 120 g of feed per day and had free access to water. Egg laying rate and cracked egg rate were recorded weekly. At the end of the study, 30 eggs from each group were selected randomly to measure egg shell strength with a FHK device (Fujihara Co. Ltd., Saitama, Japan).

Diet Composition

The basal diet was formulated according to the nutritional requirements of the flock. The diet contained 11.55 MJ/kg of ME, 16.5% CP, 3.63% calcium, 0.40% phosphorus, 0.35% methionine, and 0.95% lysine.

Bone Index and Radiography

At the end of the experiment, 20 hens were culled, weighed, and killed from each group. The left humerus, femur, and tibia were removed and cleaned of extraneous soft tissue. The bones were weighed. Bone index was calculated using the following formula: bone index = bone weight (g)/BW (kg) at slaughter. Then all bones were stored in 70% alcohol until measurements of radiographic density were made using computerized densitometry. Whole dissected bones were x-rayed with a Mikasa HF200A x-ray apparatus (44 kV and 3.88 mA-s; Mikasa X-Ray Co. Ltd., Tokyo, Japan) using Konica Medical x-ray film and Konica KR-II cassettes with intensifying screen (Konica Minolta Medical and Graphic Inc., Tokyo, Japan). Humeri, tibiotarsi, and femora were radiographed in anteroposterior views.

Twelve bones were removed from the 70% alcohol, blotted, and each placed on a 20.3 × 25.4 cm x-ray plate (in random order from each treatment). A 16-step, 0.25-mm increment aluminum step-wedge was also exposed on each plate at the same time as the bones for cross-calibration purposes. The films were digitized via a Panasonic WVBL600 monochrome video camera (with auto-gain disabled, Panasonic Corp., Tokyo, Japan) and analyzed with NIH Image v.1.62 (National Institute of Mental Health, Bethesda, MD). The NIH Image program displayed each image in false color and calibrated each x-ray from the step-wedge image in terms of millimeters of aluminum equivalence. A macro routine was written to delineate the boundary edge of each excised bone and calculate mean radiographic density for pixels contained within the boundary.
Biochemistry

Blood samples were taken throughout the study from 20 birds of each group at 2-wk intervals. Samples were centrifuged. The serum samples were harvested and frozen at −20°C for subsequent measurements of serum calcium, phosphate, and ALP concentrations. The values of calcium, phosphate, and ALP were determined by an autoanalyzer (Hitachi 7600-020, Hitachi Ltd., Tokyo, Japan).

Breaking Strengths

Breaking strengths were determined on frozen and thawed whole tibiae. These tests were carried out by 3-point bending using an Instron Universal Machine (Nørgaard-Nielsen, 1990). The center of each bone was aligned with the breaking probe (10-mm diameter), which approached at 30 mm/min with the supports for each bone placed 30 mm apart. The breaking strength was determined from the failure point (peak) of each loading curve.

Statistical Analysis

Data were analyzed by 1-way ANOVA using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL). The results were expressed as mean ± SE and differences were considered significant when \( P < 0.05 \).

RESULTS

Compared with the control, the average egg laying rate of layers in the GSK-treated group increased significantly during wk 3 to 10 (\( P < 0.05 \); Figure 1) and the percentage of cracked eggs decreased significantly during wk 2 to 10 (\( P < 0.05 \); Figure 2).

The data from postmortem BW, bone index, and radiographic measurements are given in Table 1. After 10 wk of feeding GSK, the egg shell strength, radiographic densities, and bone index of the tibia, femur, and humerus in the GSK-treated group significantly increased compared with the control group (\( P < 0.05 \)). Breaking strength of the tibia was also significantly increased in the GSK-treated group compared with the control group (\( P < 0.05 \)). Bone weight of the humerus, tibia, and femur was significantly (\( P < 0.05 \)) increased in the GSK-treated birds.

Results for biochemistry are shown in Figure 3, 4, and 5. There were significant effects of GSK on biochemical parameter. Compared with the control group, the serum concentrations of calcium and phosphorus in the GSK-treated group were decreased markedly between wk 6 to 10 (\( P < 0.05 \)), and the ALP level decreased between wk 4 to 10 (\( P < 0.05 \)).

DISCUSSION

The birds selected in this experiment were 55-wk laying hens, which is a time in the laying cycle in which osteoporosis is common in cage layers (Whitehead and Table 1. Effects of Gushukang (GSK) on BW, egg shell strength, and bone characteristics after 10 wk of treatment1

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>GSK</th>
</tr>
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<tbody>
<tr>
<td>BW (kg)</td>
<td>1.66 ± 0.03</td>
<td>1.67 ± 0.11</td>
</tr>
<tr>
<td>Egg shell strength (kg/cm²)</td>
<td>2.74 ± 0.13</td>
<td>3.27 ± 0.15*</td>
</tr>
<tr>
<td>Humerus</td>
<td></td>
<td></td>
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<tr>
<td>Bone weight (g)</td>
<td>3.74 ± 0.14</td>
<td>4.30 ± 0.18*</td>
</tr>
<tr>
<td>Bone index (g/kg)</td>
<td>2.37 ± 0.09</td>
<td>2.76 ± 0.12*</td>
</tr>
<tr>
<td>Radiographic density (mm Al)</td>
<td>1.36 ± 0.16</td>
<td>1.80 ± 0.15*</td>
</tr>
<tr>
<td>Tibia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone weight (g)</td>
<td>11.24 ± 0.43</td>
<td>12.42 ± 0.39*</td>
</tr>
<tr>
<td>Bone index (g/kg)</td>
<td>7.09 ± 0.25</td>
<td>8.08 ± 0.23*</td>
</tr>
<tr>
<td>Radiographic density (mm Al)</td>
<td>2.00 ± 0.14</td>
<td>2.47 ± 0.13*</td>
</tr>
<tr>
<td>Breaking strength (N)</td>
<td>207.14 ± 6.87</td>
<td>245.70 ± 5.11*</td>
</tr>
<tr>
<td>Femur</td>
<td></td>
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<tr>
<td>Bone weight (g)</td>
<td>9.31 ± 0.21</td>
<td>10.14 ± 0.27*</td>
</tr>
<tr>
<td>Bone index (g/kg)</td>
<td>5.94 ± 0.17</td>
<td>6.51 ± 0.18*</td>
</tr>
<tr>
<td>Radiographic density (mm Al)</td>
<td>1.97 ± 0.13</td>
<td>2.55 ± 0.07*</td>
</tr>
</tbody>
</table>

*Indicates difference (\( P < 0.05 \)) between GSK-treated group and the control.

Values are mean ± SE.

mm Al = millimeters of aluminum equivalent.
Fleming, 2000). The results from the present study demonstrated that GSK had significant effects on egg laying performance and osteoporosis in end-of-lay hens. Gushukang could significantly increase egg production and decrease the percentage of cracked eggs during the postpeak period of egg laying in hens. Radiographic density increased in the GSK-treated group compared with the control group. This increase in bone density was associated with significant increases in bone index and bone breaking strength. It seems that GSK had a protective effect on structural bone resorption. Some evidence was obtained that the concentrations of serum calcium, phosphorus, and ALP were increased in the control group during the experimental period. Analyses of biochemical markers and radiographic density confirmed the preventive effects of the GSK treatment by preventing bone loss. It was reported that decreased levels of biochemical markers are associated with the decline of bone loss (Garnero et al., 1999). In fact, some components of GSK have been shown to have effects on bone metabolism. For example, *Herbal Epimedium* enhanced osteoblastic activity, whereas it suppressed osteoclastic activity in vitro (Huang et al., 2007). *Rhizoma Atractylodis* has the effects of accelerating the circulation of blood, normalizing gastrointestinal movement, and improving the intake of nutritional ingredients, such as calcium and phosphorus (He et al., 2006). *Radix Astragali* was found to improve the proliferation and differentiation of osteoblasts and increased osteoblast osteogenic capacities in vivo (Wang and Wu, 2004).

In summary, the present study suggests that GSK could prevent the osteoporosis in end-of-lay hens. The mechanisms were possibly associated with minimizing structural bone loss and stimulating the bone mineral absorption in osteoporotic laying hens. However, it is not clear as to which components of the GSK formulation contribute to the prevention of bone loss and the mechanisms of their action.

**ACKNOWLEDGMENTS**

This work was supported by National Nature Science Foundation of China (No. 30671546).

**REFERENCES**


