Effects of Dietary Iodine and Selenium on the Activities of Blood Lymphocytes in Laying Hens

Zhigang Song, Yuming Guo* and Jianmin Yuan
State Key Laboratory for Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100094, P. R. China

ABSTRACT: The effect of dietary iodine and selenium supplementation, alone or in combination, on peripheral blood lymphocyte function was determined in laying hens. Eight-hundred-and-sixty-four New-Loman laying hens were randomly allotted into 12 dietary treatments with different inclusion levels of iodine (0, 0.1 and 0.2 mg/kg), selenium (0, 0.05, 0.1 and 0.2 mg/kg) or their combinations for 24 weeks. The lipopolysaccharide (LPS) stimulation index, concanavalin A (ConA) stimulation index, peroxide enzyme activity and phagocytosis to neutral red particles were tested. There were significant differences in LPS stimulation index, ConA stimulation index, peroxide enzyme activity and phagocytosis to neutral red particles in different iodine or selenium supplementation levels (p<0.05). The highest iodine and selenium supplementation both resulted in highest LPS-ConA-stimulation indices (p<0.05). However, when iodine was lower than 0.2 mg/kg, the additional effect of different levels of selenium did not always result in significant differences in these indices. The results indicated that iodine and selenium may affect immunity in laying hens and, when the iodine level in the laying hen is lower than 0.2 mg/kg, a selenium allowance higher than 0.1 mg/kg may be necessary to improve immunity. (Asian-Aust. J. Anim. Sci. 2006, Vol 19, No. 5 : 713-719)

Key Words: Laying Hens, Iodine, Selenium, Lymphocyte, Immune Function

INTRODUCTION

The importance of iodine as an essential element in animal’s diet arises from the fact that it is a major component of the thyroid hormone 3,5,3’ tri-iodothyronine (T₃) and 3,5,3’,5’ tetra-iodothyronine (T₄) or thyroxine (Ganong, 2001). These hormones are essential for normal growth (and physical) as well as mental development throughout the lives of animals and human and they also set the basal metabolic rate (BMR) and play active roles in digestion (Miller et al., 1974), thermoregulation, intermediary metabolism, growth, muscle function, circulation, the seasonality of reproduction and immune defense (Follett and Potts, 1990). It is well-known that the thyroid hormones have both gross and fine metabolic effects on lymphoid cells (Fabris, 1973; Lundell and Blomgren, 1976; Keast and Taylor, 1982; Li et al., 1993; Philip et al., 1995). Iodine deficiency or in excess has an effect on thyroid hormone synthesis and, consequently, may influence the function of the animal’s lymphocytes.

Selenium is an essential component of the antioxidant enzyme glutathione peroxidase (GPx) which removed potentially damaging lipid hydroperoxides and hydrogen peroxide (Anita et al., 2004). Selenium can act as an antioxidant in the extracellular space, the cell cytosol, in association with cell membranes and specifically in gastrointestinal tract, all with potential to influence immune processes (Arthur et al., 2003). In addition to its known essentiality, selenium plays an important role in the control of thyroid hormone metabolism. The thyroid gland’s major hormone product, T₄, is converted by 5’ (outer ring)-deiodination to the more biologically active hormone, T₃, by the type I and type II iodothyronine deiodinases (ID-I, ID-II). T₄ can also be converted to reverse triiodothyronine (3, 5’, 3’-triiodothyronine, γT₃), by 5 (inner ring) deiodination (Leonard and Visser, 1986). In each of the cases, free iodine is released upon conversion of these hormones. ID-I has been shown to be a seleno-enzyme (DePalo et al., 1994). More recently it was discovered that the ID-II and type III (ID-III, the inner ring deiodinase responsible for deactivating T₄ to γT₃ and T₃ to T₂) are also selenocysteine enzymes (Davey et al., 1995; Ramauge et al., 1996). Therefore, selenium may play a crucial role in the maintenance of normal thyroid physiology. For example, in a selenium- and iodine-deficient animal model, iodine supplementation alone caused irreversible thyroid gland fibrosis (Hotz et al., 1996). In human subjects with both selenium and iodine deficiencies, selenium supplementation alone caused an aggravation of iodine deficiency and hypothyroidism (Contempre et al., 1991). In rat studies, iodine deficiency or excess had reverse effect on lymphocytes’ function (Fabris, 1973), and selenium modulated the thyroxine’s metabolism. These studies demonstrated a complex interaction between iodine and selenium.

Iodine- and/or selenium-deficiency exist in many parts of the world. In some areas of China, iodine and selenium in the base diet do not meet the poultry’s requirement (Guo,
SONG ET AL.

Laying hens, which have a long lifetime for production, need a more effective immune system to maintain its high anti-infection ability (Cheng, 2004a). In order to understand better the effect of dietary iodine and selenium, individually and interactively in laying hens, a series of experiments were conducted. The results of in particular their effect on lymphocytes, are presented in this report.

**MATERIAL AND METHODS**

**Animals and treatments**

Eight-hundred-and-sixty-four New-Loman laying hens with similar body weight (±50 g; 33 weeks old) were housed in cages (three birds per cage, stainless steel) in a climatically controlled room with auto-controlled lighting. Birds had free access to water and experimental diets (Table 1) formulated with four concentrations of supplemental Se (0, 0.05, 0.1, 0.2 mg/kg from sodium selenite) and three concentrations of iodine (0, 0.1, 0.2 mg/kg from calcium iodate) in the basal diet (Table 1) containing Se 0.012 mg/kg and iodine 0.098 mg/kg. Crude protein and metabolic energy (ME) contents of the basal diet were 165 g/kg and 11.08 MJ/kg respectively, as required by the NRC (1994) for HY-line brown variety. In a factorial randomized block designed experiment with twelve treatments, birds were distributed at random to 72 groups of twelve birds each. Each diet was offered to six replicate groups. The hens were fed those diets for 24 weeks. Blood samples from six layers in each treatment were taken from the wing vein for the lymphocytes’ function test.

**Determinant of iodine and selenium in experimental diets**

Iodine and selenium content of feed samples were determined in duplicate (Table 2) according the methods of Nutrition and Food Institute Files (No. 0016, 2000) (Nutrition and Food Safety Institute of Chinese Center for Disease Control and Prevention, Being, China) and GB/T 12399-96 (China State Standard).

**Proliferation assay of peripheral blood lymphocyte**

*In vitro* peripheral blood lymphocyte (PBL) proliferation response was determined by a previously described method (Lin, 1999; Xia, 2002) with slight modification after the experiment diets were fed for 24 weeks. The heparinized (20 U/mL) peripheral blood obtained by wing-vein puncture was added to the same volume of sterile Hanks balanced salt solution (HBSS) without Ca²⁺ and Mg²⁺. The diluted blood mixture was laid over half its volume of sterile lymphocyte separation medium (density = 1.077-1.080, Academy of Military Medical Science), and separated by density-gradient centrifugation at 400 × g for 30 min at 4°C to recover PBL. PBL were collected at the interface washed with HBSS three times, after which PBL were suspended in 2 mL...
Table 3. Effect of selenium and iodine on the indices of the lymphocytes’ function

<table>
<thead>
<tr>
<th>Tests of between-subjects effects</th>
<th>LPS stimulated index</th>
<th>ConA stimulated index</th>
<th>Peroxide enzyme activity</th>
<th>Phagocytosis to neutral red particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Iodine</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Selenium* Iodine</td>
<td>0.000</td>
<td>0.000</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

* S means no significant difference (p>0.05).

sterile RPMI 1640 media with NaHCO₃ (24 mM), L-glutamine (2 mM), sodium pyruvate (1 mM), HEPES (N-2-hydroxyethylpiperazine-N’-2-ethane sulfonic acid, 10 mM), penicillin (100 U/ml) and streptomycin (0.1 mg/ml). Cells were detected by trpan blue dye exclusion using a microscope to assure 95% livability. Cell suspensions were adjusted to a final concentration of 1×10⁶ cells/ml in RPMI-1640 medium. One hundred micro-liters of cell suspension, and 100 µL RPMI 1,640 with 45 µg/mL concanavalin A (ConA) (C2613, Sigma) or 25 µg/mL lipopolysaccharide (LPS) (L3129, Sigma) were added into a 96-well plate (Costar 3599). The cultures were set up in triplicate with mitogen (stimulated with Con A or LPS). The cells were then incubated in 5% CO₂ incubator at 41°C. After 56 h incubation, 10 µL CCK-8 (Dojindo CK04-11, Japan) was added to the cell culture. The cells were incubated for four more hours, then the culture was cultured under a 4°C environment and vibrated for at least 3 minutes to stop the reaction. Read the absorbance at 450 nm via an automated ELISA reader (Bio-RAD, Model 550).

Peroxidase activity assay of peripheral blood lymphocyte

Peroxidase activity of peripheral blood lymphocyte was assessed as described by Liu (2004). An aliquot of the above cell suspensions with a final concentration of 1×10⁶ cells/ml in RPMI-1640 medium were used for this enzyme activity assay. One hundred microliters of cell suspension were added into a 96-well plate (Costar 3599) and each with a blank control which was added only with the RPMI-1640 medium. The cells were then incubated in 5% CO₂ incubator at 40°C for 2 h. Then, wash the wells with saline water. After static placement overnight, the absorbance of each well was read via UV/VIS spectrometer (Lambda Bio20, PERKIN ELMER) at 550 nm.

Statistical analysis

The mean effects of the selenium and iodine were analyzed by the method of multivariate in the general linear model (GLM) of SPSS 10.0. The multiple comparisons of the data were analyzed by one-way ANOVA of SPSS 10.0, and reported as Means±SE. The significance of differences among different groups was evaluated by Least Significant Difference (LSD) post-hoc multiple comparisons test. Significance was p<0.01 unless otherwise stated.

RESULTS

The result of the tests (Table 3) of between-subjects effects showed that the selenium and iodine affected the lymphocyte’s stimulation indexes by lipopolysaccharide (LPS) (p<0.01) and concanavalin A (ConA) (p<0.01) significantly, but not that of the peroxidase activity (p>0.05) and the phagocytic activity (p>0.05).

Cellular immunity of laying hens fed with different levels of dietary selenium and iodine was tested (Table 4, Figures 1 and 2). At low levels of selenium (basal and 0.05 mg/kg added) and low iodine (basal), their peripheral blood lymphocyte (PBL) displayed the lowest stimulation index by LPS or ConA. At the highest levels of selenium and iodine, their PBLs displayed the highest stimulation index by LPS or ConA. When supplemented with iodine at the level of 0.2 mg/kg, their PBLs showed a higher stimulation index by LPS or ConA along with a higher selenium supplementation. But when supplemented with iodine 0.1 mg/kg or without iodine, their PBLs did not show a
**DISCUSSION**

The long production cycle of laying hens subjects them...
to a number of stressful agents, including the exposure to various pathogenic microorganisms, and higher caging density (Hayirli et al., 2005). It is important for the laying hens to hold an immunocompetence to face the challenges of bacterial and viral infections. When hens were imposed with immunological challenged (such as LPS), a poor laying performance of hens might occur (Cheng, 2004b). At the cellular level, the response of lymphocytes to Con A is believed to reflect the function of the thymic-derived T lymphocytes, e.g., those associated with cell-mediated immunity (Schimizu, 1979). The response of lymphocytes to LPS mitogen stimulation is related to the function of the bone marrow-derived or B lymphocytes, e.g., those associated with antibody production and, thus, humoral immunity (Tizard, 1987). Peroxidase activity and phagocytosis activity of peripheral blood lymphocyte can be used as indication of the lymphocytes’ function (Liu, 2004).

Thyroid hormones are known to influence the function and development of lymphoid organs (Paavonen, 1982). Spleen cells from thyroxine-treated mice showed an increased primary immune response in vitro to sheep red blood cells (SRBCs) (Chen, 1980). Treatment of dwarf mice with somatotropic hormone and/or thyroxine results in reconstitution of the immune capacity (Fabris, 1971) and prolongation of their life-span. The outflow of small lymphocytes from thymus is particularly increased during treatment with thyroxine (Ernstrom and Larsson, 1966). With regard to mitogen-induced lymphocyte transformation, thyroid hormones have been shown to have either an enhancing (Keast and Tayler, 1982) or suppressing (Gupta et al., 1983) effect on the response of T cell to phytohaemagglutinin (PHA). It is known that the iodine deficiency causes a low level of T4 and T3 in poultry (Guo, 1999), pigs (Schone et al., 1988), rats (Fang, 2000) and humans (Orville et al., 2000). But the effect of selenium on the thyroid hormone metabolism is controversial. Selenium depletion resulted increased T4 but decreased T3 in rats (Shen, 1999), increased T1 in humans (Wayne, 2003) and increased T4 but decreased T1 in broilers (Guo, 1999).

In the present study, supplemented iodine or selenium alone resulted a higher levels of four indexes tested. However, the combination of iodine and selenium supplementation resulted in the highest value of all four indices. Therefore a higher iodine and/or selenium supplementation can promote the laying hens’ lymphocyte functions. This observation is in agreement with that reported by Fabris (1973), who observed that iodine deficiency had a reverse effect on the rat’s lymphocytes’ function. The result of selenium supplementation in the present verified the conclusion of Arthur (2003), who reviewed that selenium-deficient lymphocytes were less able to proliferate in response to mitogen.

Although many studies had focus on the thyroid hormones effect on the animal’s immunity, little is known regarding the importance of dietary iodine and selenium for the function of lymphocytes in laying hens. In the present study, the interactive effect between selenium and iodine on the lymphocytes’ function in laying hens indicates that, in addition to its antioxidant ability which is recognized as the selenium’s most important way to achieve its immune-promotion ability (Arthur, 2003), the selenium may also affect the laying hens’ immunity by the way of modulating the thyroid hormone metabolism.

This research documented the importance of dietary iodine and selenium in maintaining peripheral blood lymphocyte’s function in laying hens. The data implied that inadequate iodine may impair selenium nutrition. When setting selenium requirement of layers, a proper allowance of iodine should also be considered. If the iodine level in the laying hens’ diet is lower than 0.2 mg/kg, higher selenium allowance than 0.1 mg/kg may be necessary to improve the laying hens’ immunity. More work should be
done to determine the most optimal iodine and selenium supplemental levels to maintain the laying hens’ immunity function.

REFERENCES


Cheng, T. SH. 2004a. Regulation of Zinc Amino Acid Complex on supplemental levels to maintain the laying hens’ immunity function.

REFERENCES


Cheng, T. SH. 2004a. Regulation of Zinc Amino Acid Complex on supplemental levels to maintain the laying hens’ immunity function.

REFERENCES


Cheng, T. SH. 2004a. Regulation of Zinc Amino Acid Complex on supplemental levels to maintain the laying hens’ immunity function.

REFERENCES


Cheng, T. SH. 2004a. Regulation of Zinc Amino Acid Complex on supplemental levels to maintain the laying hens’ immunity function.

REFERENCES


Cheng, T. SH. 2004a. Regulation of Zinc Amino Acid Complex on supplemental levels to maintain the laying hens’ immunity function.