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Maternal Dietary Supplementation with Two Sources of Selenium Affects the Mortality and the Antioxidative Status of Chick Embryo at Different Developmental Periods

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Abstract

A total of 270 Lingnanhuang broiler breeders (40-weeks-old) were allocated into three treatments with five replicates each. Breeders were fed with basal diets (Control) or diets prepared from basal diets supplemented SS or SM at a level of 0.15 mg Se/kg diet. This study showed that feeding diet supplemented with Se to the broiler breeder significantly ($P<0.05$) decreased the mortality of chick embryo. During the late period of incubation (17 - 21 day), the mortality of the chick embryo in SM treatment was remarkably lower ($P<0.05$) than that in SS treatment. Increased glutathione peroxidase activities in the embryonic liver were observed in SS and SM treatments between days 9 and 21 of development ($P<0.05$), while no differences were noted between SS and SM treatments. Also, feeding broiler breeder with Se led to a decrease ($P<0.05$) in the contents of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in the embryonic liver. Moreover, SM is more effective than SS in decreasing the content of H_2O_2 and MDA during the late period of incubation ($P<0.05$). It was concluded that supplemented with both forms of Se in the maternal diet can improve the mortality of the embryo and its antioxidative status and organic SM shows a higher value than inorganic SS during the late period of incubation.

Keywords: Selenium; Broiler breeder; Chick embryo; Mortality; Antioxidative status

Introduction

Chorioallantoic respiration turning into pulmonary respiration characterizes the process of hatching, which accompanies a 60% accelerating of the oxidative metabolism (Visschedijk, 1968). It's also a process that can incur the overproduction of free radicals which can further lead to lipid peroxidation (LPO) since chick embryo development is associated with an amassment of polyunsaturated fatty acid in tissue lipids (Speake *et al.*, 1998). A unbalanced situation between the production rate of free radicals and antioxidant ability in the developing embryos could possibly lead to serious damages to tissues at sensitive periods during development. Thus, the integrated antioxidative systems in the developing chick embryo are crucial for its development. It has been widely accepted that selenium (Se) is a key determinant of the efficiency of antioxidative system. The traditional source of Se added to the diet of animal is sodium selenite (SS) but the most suitable form of Se for animals is organic selenomethionine (SM) (Schrauzer, 2003). In poultry, it was proved that Se in maternal diet could be transferred into eggs by hens (Payne *et al.*, 2005; Pavlovic *et al.*, 2009). Our earlier studies revealed that feeding with maternal organic Se had a greater retention of Se in the eggs and tissues of the developing embryo compared with inorganic SS (Yuan *et al.*, 2011; Wang *et al.*, 2011). It's also been covered that the maternal Se supplementation not only affects newly hatched poultry, but also has a sustained effect on the chicks during postnatal development (Pappas *et al.*, 2005).

Reduced hatchability occurred when Se in the diet was deficient and it was corrected completely by Se (Latshaw and Osman, 1974), which indicates that Se plays a key role in the developing chick embryo and adequate Se in breeder diets is essential for optimum hatchability. However, little information is available on how Se improves hatchability. Additionally, the influence of different forms of Se on the hatchability remains unclear. Some scientists reported that different sources of Se showed a similar value in regulating the hatchability (Leeson *et al.*, 2008), while Renema (2003) found that hatchability in organic Se treatment was notably higher than that in inorganic Se treatment. Hence, the first object of this study is to find out whether different forms of Se have different effects on hatchability. The second purpose is to determine at which phase during the development do the maternal Se improves the embryonic mortality.

An essential consequence of the high rates of energy

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metabolism in developing chick embryo will be a large amount of reactive oxygen species (ROS). The protection against ROS and other free radicals is brought by antioxidants such as the Se-dependent glutathione peroxidase (GPx), which plays a crucial role in antioxidative defence in cells by eliminating hydrogen peroxide (H_2O_2) and LPO generated during metabolism (Jaeschke, 1995). Malondialdehyde (MDA), one of the most critical expressions of oxidative stress caused by ROS and other free radicals, is considered as an indicator of LPO (Nordberg and Arner, 2001). Although a considerable amount of information is available on the antioxidative status in the developing chick embryo (Gaal *et al.*, 1995; Surai, 1999), there is too little information about the effect of different sources of Se in the maternal diet on the antioxidative status of the chick embryo during development. Therefore, another purpose of this research was to investigate the effects of Se in maternal diets on the antioxidative status in the developing chick embryo.

Materials and Methods

Birds and Dietary Treatments

The present trial was conducted with 270 Lingnan Yellow broiler breeders (40-wks-old) allocated to 3 treatments with 5 replicates each. Breeders were fed with basal diets (control diet) or diets prepared from basal diets supplemented SS (Sigma Chemical Co., USA) or SM (Sigma Chemical Co., USA) at a level of 0.15 mg Se/kg diet. Broiler breeders were raised in battery cages (two birds per cage) in the same house, where an ambient temperature was maintained at 27±3°C with 20 lx of light for 16 h per day. All birds were artificially inseminated weekly. All procedures in this trail were approved by the Animal Care and Use Committee of Zhejiang University.

From 40 to 48 week of age all birds received a low-Se basal diet to reduce the Se stored in their bodies. The experiment lasted for another 8 weeks after the pre-test. The basic ration was formulated based on NRC (1994) except for Se (Table 1). During the last 10 days of the experiment, 1275 eggs (85 eggs per replicate) were collected and then incubated under standard conditions (37.5°C and relative humility 55%) in a commercial incubator. Eggs from different replicates were placed at different layers in the same incubator.

Assessment of Embryonic Mortality

The dead germs taken from the incubator on the 7^{th}, 14^{th}, and 22^{nd} days were broken and breakout analyses of rejected fertile eggs were performed to assess when the deaths of the chick embryos occurred. Incandescent light was used for the candling of eggs. Embryonic mortality at early (1 - 6 day of incubation), middle (7 - 16 day of incubation), and late period (17 - 21 day of incubation) was measured based on the morphology of the developing embryo and the method of Pappas (2005). Chick embryos that had died during the early period were characterized by the absence of upper and lower beak and the presence of blood islands. Embryonic mortality that took place during the middle period was characterized by the presence of upper and lower beak but not occupying the entire inner-egg space. Occupying the entire inner-egg space by the chick embryo characterizes the third category of deaths occurred during the late period.

Analytical Procedures

On day 9, 14, 19 and 21 of incubation, 2 chick embryos from each replicate were collected and killed humanely; their livers were collected, frozen in liquid nitrogen immediately, and then stored at −80°C for subsequent analysis.

Liver from different embryos in each replicate were merged into one single sample and then homogenized (4000 rpm for 15 min at 4°C) in 9 volumes of sodium chloride (0.86%) with an Ultra-Turrax (IKA-Labortechnik, München, Germany). This process was conducted on ice and the supernatant was collected for further analysis.

The GPx activity was assessed using a GPx kit (Nanjing Jiancheng Bioengineering Company, Jiangsu, China), which was developed based on the analysis of reduced GSH in the enzymatic reaction. One unit of enzyme activity represents a decrease in GSH concentration of 1 μmol/mg protein per minute after subtraction of non-enzymatic mode at 37°C (Rotruck *et al.*, 1973). Results were expressed as units per milligram of protein.

The MDA level was determined via the method of Yagi (1994) and using a MDA kit (Nanjing Jiancheng Bioengineering Company, Jiangsu, China). Its principle is based on the intensity of the colour after treatment of the sample with thiobarbituric acid.

The content of H_2O_2 was measured using the OxiSelect Hydrogen Peroxide Assay Kit (Cell Biolabs, CA, USA). The brief assay principle is that non-fluorescent 10-acetyl-3,7-dihydroxyphenoxazine (in a presence of horseradish peroxidase) reacts with H_2O_2 with a 1:1 stoichiometry to produce highly fluorescent resorufin, which can be quantitated easily by a 96-well fluorescence microplate reader at excitation 530 nm/emission 590nm. Values of fluorescence are proportional to the H_2O_2 levels within the samples. The H_2O_2 contents in samples are measured via comparison with the predetermined H_2O_2 standard curve.

Protein concentration in homogenates was determined by using a kit produced by Nanjing Jiancheng Bioengineering Company (Jiangsu, China) based on the method of Bradford (1976).

Statistical Analysis

Results are presented as mean ± SE. Statistical analysis was carried out by SPSS 16.0 for Windows. Comparison between groups was made by one-way ANOVA followed by

the Duncan's multiple rang test. Significance was assigned as a level of $P<0.05$.

Results

The supplementation of Se in the maternal diet significantly ($P<0.05$) decreased the mortality of the chick embryo at early, middle, late, and the whole periods (Fig. 1). Different sources of Se led to notable ($P<0.05$) differences on the mortality of the chick embryo at late period of incubation but not at early, middle, and the whole periods ($P > 0.05$).

Our data also showed that remarkably increased ($P<0.05$) the activity of GPx in the embryonic liver existed in SS and SM treatments between days 9 and 21 of development (Fig. 2). However, no differences ($P>0.05$) between SS and SM treatments were showed (Fig. 2).

Supplementation of Se in the maternal diets led to a remarkable ($P<0.05$) decrease in the content of H_2O_2 in the embryonic liver (Fig. 3). Furthermore, different sources of maternal Se resulted to noticeable ($P<0.05$) differences in the H_2O_2 content in the embryonic liver at day 21 of development (Fig. 3).

Feeding broiler breeders with Se remarkably ($P<0.05$) reduced MDA production in the embryonic liver between days 9 and 21 of development (Fig. 4). The MDA content in the liver of the chick embryo was significantly ($P<0.05$) higher in SM treatment than that in other two treatments at days 19 and 21 of development but not the case at days 9 and 14 of development ($P > 0.05$) (Fig. 4).

Discussion

Chick embryonic mortality has long been a subject of biological interest as well as a problem of economic importance (Xi *et al.*, 2012). It's showed in Fig. 1 that the Se supplementation in the diet of breeders significantly decreased the mortality of the chick embryo, which is in accordance with the previous study reporting that deficient Se could lead to a reduce in hatchability (Latshaw and Osman, 1974). However, the influence of different sources of Se on the hatchability remains controversial. Leeson *et al.* (2008) reported that different sources of Se showed a similar value in regulating the hatchability, while Renema (2003) found that hatchability in organic Se treatment was significantly higher than that in inorganic Se treatment. Our research revealed that different sources of Se had no significant effect on the mortality of chick embryo at early, middle, and the whole periods. However, an interesting observation found in this research was that the mortality of chick embryo from SM treatment was markedly lower than that of SS treatment in the late period of incubation. Due to the dramatic changes in respiration and the pipping during the late period of development, ROS and other free radicals might be overproduced. Consequently, a potential reason for this result is the higher value of organic Se in improving the

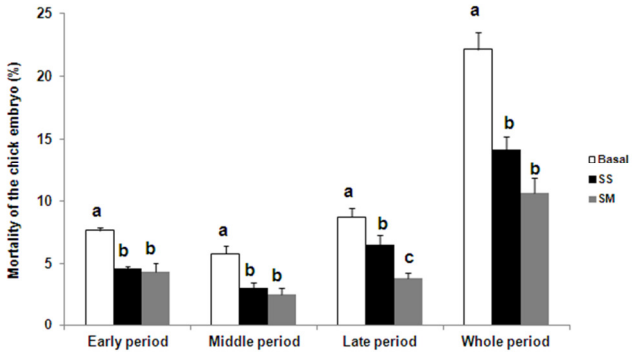

Fig. 1: Mortality of the chick embryo at different embryonic periods

Data are expressed as means ± SE (n = 5). Within each period, bars with different superscript differ significantly at $P<0.05$ by Duncan's test

SS = sodium selenite; SM = selenomethionine

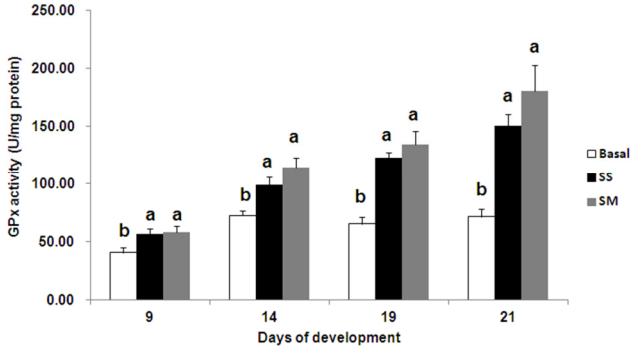

Fig. 2: Glutathione peroxidose (GPx) activity in the liver of chick embryo

Data are expressed as means ± SE (n = 5). Within each day of development, bars with different superscript differ significantly at $P<0.05$ by Duncan's test

SS = sodium selenite; SM = selenomethionine

antioxidative status protected the chick embryo from the lethal oxidative stress led by overproduced ROS and other free radicals.

Among all kinds of selenoproteins, GPx is first discovered member the best known one, which plays a key role in antioxidative defense in poultry (Surai and Dvorak, 2002a, b). Several previous investigations have showed that the activities of GPx in eggs and chicks were significantly increased by Se supplementation in maternal diets (Petrovic *et al.*, 2006; Leeson *et al.*, 2008), which make it not surprising at all that the GPx activities in the embryonic liver in our study were increased in SS and SM treatments between days 9 and 21 of development (Fig. 2). Previous research found that Se stored in hens could be deposited in their eggs and then transferred to different embryonic tissues during embryogenesis (Gaal *et al.*, 1995; Paton *et al.*, 2002). Thus, the supplementation of Se in the maternal diets has an influence on the GPx activities of the chick embryos. However, there were no differences in the activities of GPx

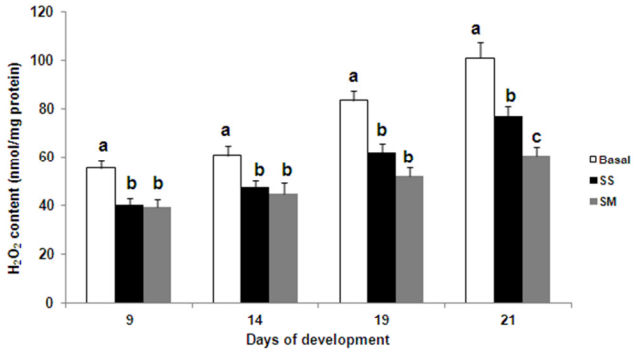

Fig. 3: Hydrogen peroxide (H_2O_2) content in the liver of chick embryo

Data are expressed as means ± SE (n = 5). Within each day of development, bars with different superscript differ significantly at $P<0.05$ by Duncan's test

SS = sodium selenite; SM = selenomethionine

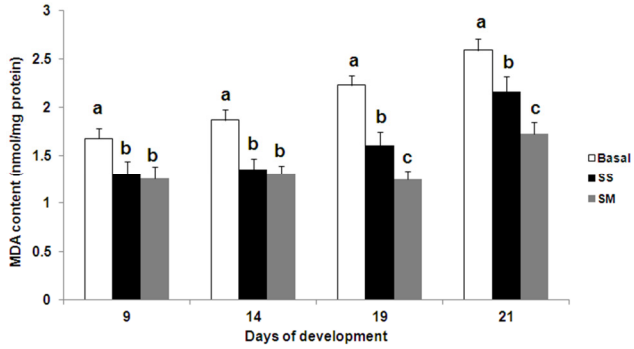

Fig. 4: Content of MDA in the liver of chick embryo

Data are expressed as means ± SE (n = 5). Within each day of development, bars with different superscript differ significantly at $P<0.05$ by Duncan's test

SS = sodium selenite; SM = selenomethionine

in the liver of the developing chick embryos existing between SS and SM treatments in this research (Fig. 2), which is in accordance with the early findings of Leeson *et al.* (2008) and Beilstein *et al.* (1988). A possible explanation for this is that Se from SM was revealed to be firstly incorporated into a wide spectrum of proteins and only incorporated into GPx later (White and Hoekstra, 1979), which may affect the availability of Se from SM for synthesis of GPx, whereas Se from SS was incorporated into GPx more efficiently (Henry and Ammerman, 1995), which can also explain that higher Se concentrations in serum and tissues were not always accompanied a corresponding higher GPx activity.

Hydrogen peroxide is an uncharged species that penetrates membranes. It's been proved that GPx plays a crucial role in removing H_2O_2 in biological systems (Michiels *et al.*, 1994), which can reduce H_2O_2 to water with the electrons coming from glutathione (Mills, 1957). In the current study, supplementation of Se in the maternal diets led to a decrease in the H_2O_2 content in the embryonic liver

Table 1: Composition of the basal diets fed to broiler breeders[1]

Ingredients	%	Composition	%
Corn	64.6	ME (MJ/kg)	11.24
Soybean meal	25.0	Crude protein	16.11
Limestone	7.0	Calcium	3.02
Monocalcium phosphate	1.8	Total phosphorus	0.65
DL-Methionine	0.3	Lysine	0.82
Salt	0.3	Methionine	0.55
Vitamin&Mineral premix[2]	1.0	Methionine + Cysteine	0.81

[1]Sodium selenite and selenomethionine were premixed in corn and added to the diets at 0.15 mg selenium per kg diet. The analyzed Se concentration (mg/kg) in the diets was as follows (n=5): basal diet, 0.058; SS-supplemented diet, 0.211; Se-Met-supplemented diet, 0.204. Results are expressed as means

[2]Provided per kilogram of diet: zinc, 72 mg; iron, 72 mg; manganese, 90 mg; copper, 7 mg; iodine, 0.9 mg; Vitamin A, 10,800 IU; Vitamin D_3, 2,160 IU; Vitamin E, 27 IU; thiamin, 1.8 mg; menadione, 1.4 mg; pyridoxine, 4.1 mg; riboflavin, 8 mg; Vitamin B_{12}, 0.01 mg; Calcium pantothenate, 11 mg; niacin, 32 mg; Biotin, 0.18 mg; Folic acid, 1.08 mg

(Fig. 3), which is consistent with the increase in the GPx activity in this experiment. What's more, different sources of Se resulted to significant differences in the H_2O_2 content in the embryonic liver at day 21 of development (Fig. 3) while no differences were found in the embryonic liver GPx activity at day 21 of development between SS and SM treatments. Unfortunately, due to the limited evidence, we cannot fully explain this.

As a metabolic product of lipid peroxides, MDA can be used as an indicator of LPO. Previous study reported that lower GPx activity is necessarily going with the increase of the concentration of MDA (Balogh *et al.*, 2004). Similar results were also found in the present study (Fig. 4), which indicates that Se enhanced the ability of the developing chick embryo to protect from oxidation. Besides, the present research also showed that feeding breeders with SM significantly reduced MDA production in embryonic liver at days 19 and 21 of development, which was not the case in the chick embryos from SS treatment (Fig. 4). Since SM can be incorporated nonspecifically into tissue proteins substitute for methionine (Schrauzer, 2000; 2003), more Se will be deposited in the chick embryo when using organic Se. Furthermore, due to the change in respiration and pipping during the late part of incubation, a higher metabolism rate is needed to produce more energy which will lead to a larger production of free radical. Accordingly, more antioxidant is required to maintain the balance of the production rate of free radical and antioxidative capacity in the developing embryo. Therefore, a logical interpretation for this phenomenon is, compared to inorganic Se, SM is more effective in improving the antioxidative status of the chick embryo because of its higher value in Se deposition.

In conclusion, both Se sources (SS and SM) could improve the mortality and the antioxidative status of the chick embryo. But during the late period of incubation, SM shows a higher value than SS in decreasing the mortality and elevating the antioxidative status of the chick embryo.

Acknowledgments

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