Protective effects of sodium selenite against aflatoxin B<sub>1</sub> on haemoglobin content, erythrocyte count, and immune adherence function in broilers

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ABSTRACT

Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) commonly found in feed stuffs causes hepatocellular carcinoma and immunosuppression. Selenium (Se) is mainly known as an important antioxidant and immunostimulant. To evaluate the protective effects of dietary sodium selenite against AFB<sub>1</sub>-induced erythocytic toxicology, one hundred male Avian broilers were randomly divided into five groups viz.: fed with basal diet (control group), 0.3mg/kg AFB<sub>1</sub> (AFB<sub>1</sub> group), 0.3mg/kg AFB<sub>1</sub> +0.2mg/kg Se (+Se group '!), 0.3mg/kg AFB<sub>1</sub> +0.4mg/kg Se (+Se group a!) and 0.3mg/kg AFB<sub>1</sub> +0.6mg/kg Se (+Se group b!), respectively. At 7, 14 and 21 days of the experiment, the red blood cells (RBC count), content of hemoglobin (Hb) and the immune adherence function were determined. Compared with control group, the number of RBC in AFB<sub>1</sub> group was increased, while the content of Hb and erythrocyte rosette rates in AFB<sub>1</sub> group were decreased significantly. Dietary sodium selenite, however, could decrease the number of RBC; increase the content of Hb and erythrocyte rosette rate significantly. It was indicated that sodium selenite in the diets could exert protective effects against AFB<sub>1</sub>-induced damage on the red blood cells, and the transport capacity and immune adherence function of erythocyte could be maintained.

Key words: Aflatoxin B<sub>1</sub>, Broiler, Haemoglobin, Red Blood Cell, Rosette rate, Sodium selenite.

INTRODUCTION

Aflatoxins, a class of mycotoxins, are produced by filamentous fungi particularly by certain strains of Aspergillus flavus and Aspergillus parasiticus (Hussain et al. 2010). Among various aflatoxins, Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is the most potent carcinogen. Aflatoxicosis causes several injuries in organs and tissues, the decrease in growth rate, the increase in death rate, immunosuppression, anemia, and increase in coagulation time and deteriorates lipid, carbohydrate, and protein metabolism (Raju and Devegowda 2000). Thus, consumption of aflatoxin-contaminated food by human and animals causes important health problems (Dönmez et al. 2012). Antioxidant nutrients, including Se, can protect against the damages caused by harmful agents, especially free radicals (Keshacarz et al. 2001). Potential selenium-mediated health benefits include delay of aging, functioning of the immune system, and prevention of certain forms of cancer (Steinbrenner and Sies 2009). Erythrocytes are highly specialized cells which serve as carrier of gases between lungs and tissues (Verma and Raval 1992). Besides respiration function, it has been elucidated that the nucleated erythrocytes, like those of birds, may have a direct role in the immune response. So far, the effects of disease and nutrients on erythrocyte immune function have been reported (Deng et al. 2013). Though the protective effects of Se against AFB<sub>1</sub> have been observed (Chen et al. 2013), the effects of Se on function of red blood cells are hardly reported, especially the immune function of red blood cells. The present research was conducted to detect the effects of dietary sodium selenite against AFB<sub>1</sub> on the number of red blood cells, content of haemoglobin and erythrocyte immune function in broilers.

MATERIALS AND METHODS

Chickens and diets: One hundred 1-day-old healthy male Avian broilers were obtained from a commercial rearing farm (Wenjiang poultry farm, Sichuan province). Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) purchased from Fermentek Ltd. (Jerusalem, Israel, 1162-65-8) and dissolved in methanol completely was mixed into the corn-soybean basal diet to produce the diets of experimental groups. The equivalent methanol was mixed into corn-soybean basal diet. The broilers were divided into five groups and fed on diets as follows: basal diet (control group), 0.3mg/kg AFB<sub>1</sub> (AFB<sub>1</sub> group), 0.3mg/kg AFB<sub>1</sub> +0.2mg/kg Se (+Se group !), 0.3mg/kg AFB<sub>1</sub> +0.4mg/kg Se (+Se group a!) and 0.3mg/kg AFB<sub>1</sub> +0.6mg/kg Se (+Se group b!), respectively.
AFB₁ + 0.4mg/kg Se (+Se group aII) and 0.3mg/kg AFB₁ + 0.6mg/kg Se (+Se group b!). Broilers were housed in cages with electrically heated units and were provided with water as well as aforementioned diets *ad libitum* for 21 days. Nutritional requirements were adequate according to National Research Council (NRC, 1994) and Chinese Feeding Standard of Chicken (NY/T33-2004). All procedures of the experiment were performed in compliance with laws and guidelines of Sichuan Agriculture University animal welfare institute.

**Blood Samples:** At 7, 14 and 21 days of age, 1 mL blood samples were collected by vein puncture (jugular vein) from 5 birds in each group in anticoagulant coated test tubes (40 g/L of Ethylene Diamine Tetraacetic Acid disodium salt).

**Count of red blood cells (RBC) and content of haemoglobin:** The RBC count and Hb were determined according to the methods described in reference (Ma, 2004).

**C₃b receptor rosette rate (C₃bR RR) and immune complex rosette rate (ICR):** Yeasts and complement-coated yeasts were purchased from Immunology Department Shanghai Changhai Hospital, and used for determining C₃bR RR and ICR respectively. The methods were as described in reference (Guo, 1982).

**Statistical analysis:** The results were showed as means ± standard deviation (M ± SD). Statistical analysis was performed by using one-way ANOVA test of SPSS 16.0 software. The difference between groups was considered significant when a probability (P) was < 0.05.

**RESULTS AND DISCUSSION**

Hematological indices can be used to indicate physiological, pathological and nutritional status of an animal (Toghyani et al. 2010). Compared with control group, the number of RBC in AFB₁ group was markedly increased (P<0.01 or P<0.05), while the content of Hb was significantly decreased (P<0.01) (Table 1). The results were in accordance with a previous research, in which the RBC count was significantly increased and the Hb significantly decreased in lambs ingesting 2.5 mg AFB₁/kg diet (Fernandez et al. 1996). According to the results, the RBC count was negative correlated with Hb content. This situation often occurs in earlier stage of iron-deficiency anemia (Pasricha et al. 2010), and the decrease of serum iron ion level has been observed in lambs fed with AFB₁ (Fernandez et al. 1996). Aflatoxin-related anemia has been reported in rats (Fernandez et al. 1996), CD-1 mice and cattle (Brucato et al. 1986). Through stimulating phospholipid A₁ to initiate lipid peroxidation in cells, AFB₁ metabolites could disturb the integrity of erythrocytic membrane and accordingly induce the decreased erythrocyte deformability and increased erythrocyte viscosity (Shen et al. 1995). Due to iron-deficiency anemia and viscous erythrocytes, the transportation capacity of erythrocytes in chicken was impaired by AFB₁ (Table 2).

Recent evidence suggests that erythrocytes are natural immunity cells and the C₃b receptor (CR₃) on erythrocyte plays an important role in the efficient transport of antigen-antibody-complement complexes for clearance by the fixed macrophage system (Cohen et al. 1999). The C₃b Receptor Rosette Rate (C₃bR RR) is closely associated with the quantity and activity of CR₃ on erythrocyte membranes (Paccaud et al. 1990), and the Immune Complex Rosette Rate (ICR) is associated with the content of circulating immune complex (CIC). Our results showed that the C₃bR RR and ICR in AFB₁ group were markedly lower (P<0.01) than that in control group from 7 to 21 days of age (Table 2). There are two types of CR₁ distribution on erythrocyte membrane: disperse and cluster, but only the cluster of CR₁ can bind to complement strongly. The primary lesion of erythrocyte immune function is due to the decreased number or structural

| TABLE 1: Effect of sodium selenite against aflatoxin on the numbers of red blood cells and the content of haemoglobin in broilers. |
|-----------------|---------------|---------------|---------------|
|                  | 7days         | 14days        | 21days        |
|                  | 7days         | 14days        | 21days        |
| RBC count (1×10¹⁰/L) |               |               |               |
| Control group    | 173.30±12.84b | 220.10±18.28bc| 240.90±15.81b |
| AFB₁ group       | 226.60±15.95ade| 269.60±17.29ade| 264.90±16.47ade|
| +Se group a!     | 187.90±13.47ade| 257.20±12.63ade| 256.60±10.94ad |
| +Se group b!     | 169.80±10.48bc| 216.60±14.82bc| 228.85±19.54bc |
| Control group    | 170.00±7.74bc | 222.30±16.85bc| 239.10±14.40bc |
| haemoglobin (g/100mL) |               |               |               |
| Control group    | 7.49±0.25bc   | 7.54±0.39bc   | 9.86±0.47bc   |
| AFB₁ group       | 6.42±0.33ade  | 6.12±0.45ade  | 8.01±0.53adce |
| +Se group a!     | 6.59±0.44ade  | 6.82±0.33ade  | 10.15±0.62b   |
| +Se group b!     | 7.58±0.24ade  | 7.66±0.48bc   | 10.92±0.64ab  |

Data are presented with the means ± standard deviation (n=5). Letters A, B, C, D and E represent the significant difference (P<0.01) between the group and control group, AFB₁ group, +Se group I, +Se group II, +Se group III, respectively. Letters a, b, c, d and e represent difference (P<0.05) between the group and control group, AFB₁ group, +Se group I, +Se group II, +Se group III, respectively.
change of CR<sub>s</sub>, and the secondary lesion is attributed to the binding site occupied by CIC. In present research, the C<sub>b</sub>RR and ICR in AFB<sub>1</sub> group were lower than those in control group, showing as the primary lesion. It has been demonstrated that AFB<sub>1</sub> could induce oxidative stress and gradually impair membrane system (Shen et al.1995). So, AFB<sub>1</sub>-induced membrane damage led to the impairment of CR<sub>s</sub> receptor, and the immune adherence function of erythrocyte could be accordingly reduced.

However, Se supplied into the diets could reduce the AFB<sub>1</sub>-induced damage on erythrocyte. Compared with AFB<sub>1</sub> group, the numbers of RBC in three +Se groups were decreased to be close to that in control group, especially in +Se group a! and b!, while the contents of Hb were increased (Table 1). The C<sub>b</sub>RR and ICR in AFB<sub>1</sub> group were lower than those in AFB<sub>1</sub> group (Table 2). The results may result from antioxidative abilities of Se, which is essential for cell survival in environments containing peroxides (Surai et al.2002). Although AFB<sub>1</sub> may reduce the abilities of antioxidant enzymes against ROS (Choi et al.2010), Se supplementation in the diet improves the activities of GSH-Px, enhances mechanisms of selenium-dependent and selenium-independent ROS scavenging, while H<sub>2</sub>O<sub>2</sub> and lipid hydroxides were reduced to less reactive products and antioxidative capacity was restored, consistent with the results on antioxidative abilities of spleen in this research (Wang et al.2013). The reduction of reactive oxygen metabolites by GSH-Px helps to maintain membrane integrity. As a result, the AFB<sub>1</sub>-induced damage of erythrocyte could be relieved by dietary Se and the CR<sub>s</sub> on the membrane of erythrocytes could be protected from impairment induced by AFB<sub>1</sub>, which was partially contributed to these results. Moreover, the relationship between iron-deficiency anemia and selenium-deficiency has been reported (Gürgöze et al.2006), and selenium is recommended to be added to the treatment of iron-deficiency anemia (Gürgöze et al.2004). Thus, Se supplementation may exert the protective effects on erythrocyte by alleviating harmful influence of iron-deficiency anemia caused by AFB<sub>1</sub>.

In conclusion, 0.3mg/kg AFB<sub>1</sub> could induce decreased contents of Hb and C<sub>b</sub>RR and ICR, increased number of RBC, and the erythrocyte functions were impaired. On the contrary, Se supplied into the dietary could reduce the AFB<sub>1</sub>-induced lesions on erythrocyte by increasing contents of Hb and C<sub>b</sub>RR and ICR, and decreasing the number of erythrocyte. The positive effects of Se against AFB<sub>1</sub> may be associated with antioxidative capacity of Se.

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**REFERENCES**


