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Effects of deoxynivalenol exposure on cerebral lipid peroxidation, neurotransmitter and calcium homeostasis of chicks *in vivo*

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ABSTRACT

During current research, the effects of deoxynivalenol (DON) exposure on cerebral lipid peroxidation, neurotransmitter secretion and calcium homeostasis in chicks were evaluated. One hundred and twenty Hailan chicks (male, 1-day-old) were randomly divided into four groups. Chicks in low, medium and high dose groups were fed with 0.27, 1.68 and 12.21 mg/kg^{-1} DON respectively by gavage according to feed intake. Chicks in control group were fed with physiological saline by gavage. The trials were conducted for 36 d. At the end of the trials, twenty chicks per group were sacrificed, and the cerebra were collected for measuring the brain indices. Compared with the control group, the activities of total superoxide dismutase (T-SOD) and glutathione peroxidase were significantly decreased in treatment groups ($P < 0.05$), the contents of malondialdehyde in high dose group were increased ($P < 0.05$), the catalase activities and nitric oxide contents in medium and high dose groups were decreased ($P < 0.05$), and the activities of T-AOC in high dose group were reduced ($P < 0.05$). Compared with the control group, the concentrations of norepinephrine and 5-hydroxytryptamine in high dose group were obviously increased ($P < 0.05$), while the concentrations of dopamine were decreased ($P < 0.05$). Meanwhile, the concentrations of calcium and calmodulin (CaM) in medium and high dose groups were lower than those of the control group ($P < 0.05$), and the gene relative expression of CaM mRNA in treatment groups were significantly reduced ($P < 0.05$), in a dose-dependent manner. These results suggested that DON exposure can affect the cerebral lipid peroxidation, neurotransmitters secretion and the balance of calcium homeostasis in chicks.

1. Introduction

Mycotoxins are toxic secondary metabolites that have similar biological activities, produced by organisms of the fungus kingdom (Ren et al., 2015; Hussein and Brasel, 2001). Many mycotoxins have been identified. Deoxynivalenol (DON) is the most commonly detected trichothecene mycotoxin produced by *Fusarium graminearum* and *Fusarium culmorum*, and it is ubiquitously found in many food and agricultural commodities such as wheat, maize, barley, oats and corn (Ma et al., 2012; Wegulo, 2012; Pestka and Smolinski, 2005). It has been reported that DON produced extensive contaminations in many countries worldwide (Kimanya et al., 2014; Mudili et al., 2014; Nordkvist and Häggblom, 2014; Brera et al., 2013). DON-contaminated feed could induce diverse toxicities in animals, including anorexia, nausea, diarrhea, emesis, vomiting, fever, and slow response (Wang et al., 2016; Sobrova et al., 2010).

Although some studies suggested that DON can be slightly tolerated by adult poultry, toxicity of DON in chicks should not be overlooked, especially DON damage to the nervous system, which results loss of appetite, growth retardation, depression, and other related diseases (Ebrahem et al., 2014; Geng et al., 2015). Meanwhile, DON can reach up to peak concentration quickly through the blood-brain barrier, but most are metabolized rapidly and less than 1% is absorbed in chicks exposed to DON (Yunus et al., 2010). In addition, many studies suggested that the toxicity of DON was closely related with lipid peroxidation. DON could cause obvious lipid peroxidation in animal organism and cultured cells *in vitro*, suppress the activities of antioxidant enzymes, and then trigger oxidative stress (Pestka and Smolinski, 2005;

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Borutova et al., 2008; Krishnaswamy et al., 2010). The invasion of brain tissues were susceptible to free radicals which led to lipid peroxidation because of the high concentrations of unsaturated fatty acids and catecholamine and high levels of oxidative metabolism capability in brain tissue. However, the effect of DON on lipid peroxidation of brain tissue in poultry is unclear.

Neurotransmitters are important information transducers in central nervous system, with a wide range of biological activities. Changes in neurotransmitter level directly affect the organism's behavior and activities, and are closely associated with many diseases (Barr et al., 1993). Studies have shown that DON can affect the secretion of neurotransmitters in the brain, which showed that the increase of norepinephrine (NE) and 5-hydroxytryptamine (5-HT) and the decrease of dopamine (DA) in different parts of brain tissue in piglets, and resulted in immune response lag (Prelusky et al., 1992). Moreover, DON affected 5-HT secretion and led to food refusal and slow growth in turkey fed with DON-contaminated feed (Girish et al., 2008). But the effect of DON on neurotransmitter secretion in chicks has not been clearly understood.

Calcium homeostasis is a major factor in maintenance of cell integrity and function. Calcium is an important second messenger and regulator of cell homeostasis (Plank et al., 2006). Calmodulin (CaM) is a highly conserved calcium-binding protein that transduces calcium signals into downstream effects, influencing a wide range of cellular processes, including calcium homeostasis. Besides, the cellular calcium homeostasis disruptions during oxidative stress may cause serious damage or death. It is reported that, when the DON exposure concentrations increased, the intracellular calcium concentration and CaM mRNA levels of splenic lymphocytes isolated from chicks gradually increased in a dose-dependent manner (Ren et al., 2016). However, very few studies have shown the toxicity of DON in deregulation of calcium homeostasis *in vivo*.

At present, the underlying mechanisms of DON on the neurotoxicity in poultry have not been completely elucidated. The primary objective of the present study was to determine the effect of different doses of DON on cerebral lipid peroxidation, neurotransmitters secretion and calcium homeostasis changes in chicks.

2. Materials and methods

The experimental use of animals and procedures followed were approved by the Anhui Agricultural University Animal Care Committee (ZXD-P2017625).

2.1. Chemical and reagents

All chemicals used in this study were of highest rating and purity. DON (CAS NO. D0156-1MG), paraformaldehyde, norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindole-3-acetic acid (5-HIAA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Catalase (CAT), total superoxide dismutase (T-SOD), total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), nitric oxide (NO), and calmodulin (CaM) ELISA kits were obtained from Nanjing SenBeiJia Biological Technology Co., Ltd (Nanjing, China). Calcium Assay kit was purchased from Beijing New Jiyuan Biotechnology Co., Ltd (Beijing, China). Trizol reagent was purchased from Invitrogen Biotechnology Co., Ltd. (Shanghai, China). SYBR PremixScript RT-PCR Kit II was purchased from TaKaRa (Shiga, Japan).

2.2. Animals and diets

One hundred and twenty 1-day-old healthy male Hailan chicks were obtained from a commercial rearing farm (Anqin poultry farm, Anhui province) at day of hatching. Thirty chicks were kept in each group, which was equipped with wood shreds and were reared with lighting regimen 23 h light and 1 h dark. The initial room temperature of 32–33 °C was reduced weekly by 1 °C to a final temperature of 28 °C. The relative humidity was within a range of 50–60%. The chicks provided with water as well as *ad libitum* feed only a basic commercial diet. Nutritional requirements were adequate according to National Research Council (NRC, 1994) and the Chinese Feeding Standard of chicks (NY/T33-2004). The compositions and nutrient levels of basal diet are shown in Table 1.

Table 1
Ingredients and nutrient levels of experimental diets.

Ingredients, %	Levels	Nutrient levels[b], %	Levels
Corn	58.00	Metabolizable energy[c]	13.27
Soybean meal	24.00	Crud protein	22.95
Fish meal	12.00	Ca	1.00
Vegetable oil	3.00	P	0.50
L-Lysine	0.15	Lysine	1.52
DL-Methionine	0.20	Methionine	0.6
Limestone	0.85	Cystine	0.25
$CaHPO_4$	0.35		
Additives[a]	1.45		

[a] Additives provided the following per kg of diets: VA 12500 IU, VD_3 2500 IU, VK_3 2.65 mg, VB_1 2 mg, VB_2 6 mg, VB_{12} 0.025 mg, VE 30 IU, Cu 8 mg, Zn 75 mg, Fe 80 mg, Mn 100 mg, Se 0.15 mg, I 0.35 mg, biotin 0.0325 mg, folic acid 1.25 mg, pantothenic acid 12 mg, nicotinic acid 50 mg.

[b] Nutrition levels in the parentheses were measured values (air-dry basis), while the others were calculated values.

[c] The unit of metabolizable energy is MJ/kg.

2.3. Study design and samples collection

All chicks were allowed to acclimate for one week before being randomly divided into four groups. Chicks in low, medium and high dose of groups were gavaged with 0.27, 1.68 and 12.21 mg/kg^{-1} DON according to weekly feed intake, respectively. Chicks in control group were fed with physiological saline by gavage. Perfusion dose of DON was according to that previously reported by Yunus et al. (2012). The trials were conducted for 36 days and the growth status of chicks was observed every day. All chicks in treatment group were fed with DON by gavage each week successively, a total of 5 times. After DON treatment for 5 times, all chicks were fed 1 day again and the samples were collected.

At the end of the trials, twenty chicks were randomly selected from each group, and then anaesthetized with an intramuscular injection of 846 anesthetic mixture (haloperidol, dihydroetorphine, and 2, 4-dimethylaniline thiazole) (Duan and Zhu, 2005) using doses of 0.8 mL/kg body weight, respectively. After laparotomy, the whole cerebrum was removed from chick and divided into 3 parts. Part of the left half of the cerebrum tissue was put in 1.5 vol of tissue lysates (a mixture of 0.60 mol/L perchloric acid, 0.50 mmol/L ethylenediaminetetraacetic acid diT-SODium salt and 0.1 g/L L-cysteine) to obtain homogenates and for 30 min centrifuged at 10 000 rpm, stored at −80 °C for detection of the neurotransmitter levels. Anterior part of the right half cerebrum tissue was mixed with 2 vol of phosphate-bufferd saline (PBS, pH 7.2) to obtain homogenates which were centrifuged at 10 000 rpm for 30 min at room temperature. The supernatant was then stored at −20 °C for measuring the oxidative and antioxidative indices, as well as the concentrations of calcium and CaM. The Posterior part of right half of the cerebrum tissue was stored in liquid nitrogen for measuring the relative expression levels of *CaM* mRNA.

2.4. Determination of the oxidative and antioxidative indexes

The supernatant of cerebrum tissue homogenate preserved in −20 °C was recovered to normal temperature. The activities of CAT, T-SOD, T-AOC and GSH-Px, and the concentrations of MDA and NO were