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Assessment of 28 trace elements and 17 amino acid levels in muscular tissues of broiler chicken (*Gallus gallus)* suffering from arsenic trioxide☆

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ABSTRACT

The contents of 28 trace elements, 17 amino acid were evaluated in muscular tissues (wings, crureus and pectoralis) of chickens in response to arsenic trioxide (As_2O_3). A total of 200 one-day-old male Hy-line chickens were fed either a commercial diet (C-group) or an As_2O_3 supplement diet containing 7.5 mg/kg (L-group), 15 mg/kg (M-group) or 30 mg/kg (H-group) As_2O_3 for 90 days. The elements content was analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Under As_2O_3 exposure, the concentration of As were elevated 8.87–15.76 fold, 7.93–15.63 fold and 5.94–12.45 fold in wings, crureus and pectoralis compared to the corresponding C-group, respectively. 19 element levels (lithium (Li), magnesium (Mg), aluminum (Al), silicon (Si), kalium (K), vanadium (V), chromium (Cr), manganese (Mn), nickel (Ni), copper (Cu), selenium (Se), strontium (Sr), molybdenum (Mo), cadmium (Cd), tin (Sn), antimony (Sb), barium (Ba), mercury (Hg) and lead (Pb), 9 element levels (K, Co, Ni, Cu, As, Se, Sr, Sn, Ba and Hg) and 4 element levels (Mn, cobalt (Co), As, Sr and Ba) were significantly increased ($P < 0.05$) in wing, crureus and pectoralis, respectively. 2 element levels (sodium (Na) and zinc (Zn)), 5 element levels (Li, Na, Si, titanium (Ti and Cr), 13 element levels (Li, Na, Mg, K, V, Cr, iron (Fe), Cu, Zn, Mo, Sn, Hg and Pb) were significantly decreased ($P < 0.05$) in wing muscle, crureus and pectoralis, respectively. Additionally, in crureus and pectoralis, the content of total amino acids (TAA) was no significant alterations in L and M-group and then increased approximately 10.2% and 7.6% in H-group, respectively ($P < 0.05$). In wings, the level of total amino acids increased approximately 10% in L-group, whereas it showed unchanged in M and H-group compared to the corresponding C-group. We also observed that significantly increased levels of proline, cysteine, aspartic acid, methionine along with decrease in the tyrosine levels in muscular tissues compared to the corresponding C-group. In conclusion, the residual of As in the muscular tissues of chickens were dose-dependent and disrupts trace element homeostasis, amino acids level in muscular tissues of chickens under As_2O_3 exposure. Additionally, the response (trace elements and amino acids) were different in wing, thigh and pectoral of chick under As_2O_3 exposure. This study provided references for further study of heavy metal poisoning and may be helpful to understanding the toxicological mechanism of As_2O_3 exposure in muscular tissues of chickens.

1. Introduction

Chicken meat is one of the main human diet due to low price, rich sources of proteins, vitamins, minerals and fibers. It is known that roxarsone was used as a feed additive for poultry growth promotion, efficiency of feed utilization and disease-control agents, which contributes to the exposure of consumers to arsenic (As) through the diet. The recommended roxarsone dosages are 25–50 ppm in poultry feed and approximately 70% of the broiler production units use roxarsone in the feeds in the United States (Chapman and Johnson, 2002; Wang et al., 2016). At a mean level of chicken consumption (60 g/person/day), people may ingest in the range of 72.0–85.1 μg As/person/day from chicken alone (Shah et al., 2009). Nachman et al. (2013) have reported that As-based drugs were used in poultry production and lead to residual As in chickens meat. Gul et al. (2013) have reported that As residues in different tissues (leg, breast, liver and heart muscles) of chickens were 2–10 fold higher than safe limit from five poultry farms. The content of roxarsone in poultry could reach to 21.6 mg/kg in terms

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of As (Yao et al., 2006). Due to the wide use of As in animal husbandry, people measured elemental concentrations, including As, Cd, Hg and Pb in breast muscle, and are increasing concerned about adverse effects on public health (Bond et al., 2015).

The excessive As has toxicity in nervous, skeletal, circulatory, enzymatic, endocrine and immune system. Epidemiological studies have shown that As exposure causes oxidative stress, which leads to oxidize amino acids damaging to the cellular membrane and tissue in rat (Agrawal et al., 2015). As toxicity mostly results from its ability to interact with sulfhydryl groups of proteins and enzymes, which may alter cellular redox status and eventually lead to cytotoxicity (Hughes, 2002). Moreover, As can replace the former in energy transfer phosphorylation reactions due to its similar biochemical properties to phosphate, resulting in the impairment of ATP synthesis (Fattorini and Regoli, 2004). In this sense, there have been many hypotheses that As can induce cellular necrosis or apoptosis, inflammatory response, change the signaling model and so on (Basu et al., 2001). Notably, the balance of amino acid and elements are essential for normal functioning of animals. It has various beneficial roles: regulates the nerve impulses and helps to maintain muscle tone; increases the levels of growth hormones; maintains normal enzymes activities like oxygen transport, electron transfer and gene regulation; and is effective in free radical scavenging function (Dong et al., 2016; Elseweidy et al., 2010; Kaluev and Nutt, 2007). Previous data have clearly reported that the concentrations of As was increased dose-dependently and seemed to be distributed in all of the tissues of rats (Cui and Okayasu, 2008). As exposure can disturb the homeostasis of essential trace minerals and altered the expression of cysteine/glutamate transporters in mouse brain (Ramos-Chávez et al., 2016). On the other hand, As exposure can inhibit amino acids synthesis pathways and leads to phytotoxicity. Amino acid plays important roles in As exposure through osmotic adjustment and the accumulation of compatible osmolytes, detoxification of ROSs and pH regulation (Tripathi et al., 2014). Li et al. (2017a,b) have noted that heavy metal can be absorbed by organisms and bioaccumulate, which increasing the adverse health effects. Previous studies have reported that deficiency of elements (K, Mg, Ca, Zn and Se) were significantly correlated with a number of amino acids such as methylhistidine, alanine, isoleucine and phenylalanine (Gok et al., 2016). Jiang et al. (2016) have demonstrated that heavy metal could induce oxidative damage, which could cause the high cooking loss and high discoloration rate, decreasing the muscle quality (amino acid, fatty acid) in fish. Under heavy metal exposure, increased levels of proline correlate with enhanced metal tolerance in a transgenic alga (Siripornadulsil et al., 2002). Additionally, Bhatia et al. (2005) have showed that alanine, aspartic acid and glutamic acid levels are increased in response to the nickel in xylem. Glutamic acids, cysteine and glycine exhibiting an increasing trend which showed the detoxifying capacity in grasshopper under As exposure. Yao et al. (2013a), (2013b) have reported that the cysteine plays important roles in the antioxidant function of chickens myoblasts. These studies suggest that toxicity effects of As connected with trace elements and amino acids.

In chickens, wings, crureus and pectoralis are primary muscular tissues consumption for people, play an irreplaceable role in maintaining organism in a favourable environments. Prolonged As ingestion leads to its smaller accumulation in muscle because the organs is rich in oxidative enzyme systems (Benramdane et al., 1999). Several drugs are on the market, which are known to cause skeletal muscle toxicity. However, litter is known on the alterations in 28-trace elements and amino acid in muscular tissues of chickens in response to As exposure. Therefore, the objective of this study was to determine if the residual of As in the muscular tissues of chickens were dose-dependent under arsenic trioxide (As_2O_3) exposure. Another objective was to investigate the effect of As_2O_3 on 28-trace elements and 17 amino acid levels in muscular tissues of chickens, and helping to discover the disturbed metabolic pathways responsible for As toxicity. This study provided references for further study of heavy metal poisoning and may be helpful to understanding the toxicological mechanism of As_2O_3 exposure in muscular tissues of chickens.

2. Materials and methods

2.1. Animals and experimental design

Two-hundred male Hy-line chickens (one-day-old; Weiwei Co. Ltd., China) were housed in the Institutional Animal Care and Use Committee of Northeast Forestry University (Harbin, China) (approval no. UT-31; 20 June 2014). Chickens were randomly divided into four groups (50 chickens per group). Because this study focuses on the toxicological effects of As, rather than the arsenic-laden residuals in the environment, the concentrations of As_2O_3 in used in this study was determined not based on the actual residual concentration in the environment. To observe the dose-dependent dynamic change, we set the four groups of different dose levels: a control group (0 mg/kg BW, C-group), a low As_2O_3-treated group (0.625 mg/kg BW, corresponding 7.5 mg/kg feed, L-group), a middle As_2O_3-treated group (1.25 mg/kg BW, corresponding 15 mg/kg feed, M-group), a high As_2O_3-treated group (2.5 mg/kg BW, corresponding 30 mg/kg feed, H-group), which represent zero, one-eightieth, one-fortieth and one-twentieth of the median lethal dose (LD_{50}) (50 mg/kg) for chickens, respectively. The dose applied in the present experiment was illustrated in our previous publication (Li et al., 2017a, 2017b; Zhao et al., 2017). The composition of the diet and water was summarized in Table 1. Each group of chickens was housed separately from the other to avoid the cross exposure of As_2O_3. The duration of the experiment was 90 days. The chicken were fed in battery cages and received identical standard feeding and management, with housing and care of chickens conforming to the guidelines of the Institutional Animal Care and Use committee of Northeast Forestry University. The experimental animals were housed in an environmentally controlled room with a temperature of 23 ± 2 °C and a relative humidity within the range of 40–70%. The air was changed 10–15 times per hour. The light was set for a 12 h light and dark cycle. All chickens were examined for clinical signs of ill health and observed during the experiment. Throughout the entire experimental period, chickens were allowed ad libitum consumption of feed and water. Fifty chickens in each group were selected at 90 days of the experiment and euthanized with sodium pentobarbital. The wings, crureus and pectoralis were quickly excised and blotted. The tissues were rinsed with ice-cold 0.9% NaCl solution, frozen immediately in liquid nitrogen, and stored at −80 °C until required. All procedures and

Table 1
The composition of the basal diet and water.

Basal diet	Concentration (g/kg)	Water	Concentration (mg/L)
Corn	658.7	Arsenic	0.01
Soybean meal	50.0	Cadmium	0.005
Cottonseed meal	80.0	Chromium	0.05
Rapeseed meal	60.0	Lead	0.01
Limestone	85.5	Mercury	0.01
Dicalcium phosphate	15.0	Selenium	0.01
Salt	2.0	Aluminum	0.2
$NaHCO_3$	2.0	Ferrum	0.3
Choline chloride	1.0	Copper	1.0
Distillers dried grains with solubles	40.0	Zinc	1.0
65% Lysine	4.1		
Premix *	0.4		
DL-Methionine	1.3		
Threonine	0.5		

Premix *: Supplied the following per kilogram of complete diet: VA,4.4 mg; cholecalciferol, 40 μg; DL-α-tocopheryl acetate, 10.0 mg; vitamin K_3,3.0 mg; vitamin B_{12}, 10 μg; thiamine, 3.5 mg; riboflavin, 8.0 mg; pyridoxine, 4.5 mg; folic acid, 1.0 mg; pantothenic acid, 5.0 mg; biotin, 0.02 mg; Se (as $NaSeO_3$), 0.3 mg; and I (as KIO_3), 0.4 mg.