RESEARCH ARTICLE



# Simultaneous analysis 26 mineral element contents from highly consumed cultured chicken overexposed to arsenic trioxide by inductively coupled plasma mass spectrometry

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Received: 19 May 2016 /Accepted: 22 July 2016 / Published online: 13 August 2016  $\oslash$  Springer-Verlag Berlin Heidelberg 2016

Abstract This study assessed the impacts of dietary arsenic trioxide  $(As<sub>2</sub>O<sub>3</sub>)$  on 26 mineral element contents in the liver and kidney of chicken. A total of 100 male Hy-line cocks were randomly divided into 2 groups (50 chickens in each group), including an arsenic-treated group (basic diet supplemented with  $As<sub>2</sub>O<sub>3</sub>$  at 30 mg/kg) and a control group (basal diet). The feeding experiment lasted for 90 days and the experimental animals were given free access to feed and water. We determined 26 mineral elements in the liver and kidney by inductively coupled plasma mass spectrometry (ICP-MS). The results showed that nine element levels (Al, Mn, Co, Cu, Zn, Se, Cd, Ba, and Pb) were significantly decreased ( $P < 0.05$ ) in the liver of chickens exposed to  $As<sub>2</sub>O<sub>3</sub>$  compared to the control chickens where three element levels (Ni, As, and Hg) increased significantly ( $P < 0.05$ ). The results in the kidney showed that nine element levels (Al, K, Ca, Cr, Mn, Ni, Sb, Ba, and Pb) were significantly decreased ( $P < 0.05$ ) in the chickens exposed to  $As_2O_3$  compared to the control chickens where four element levels (Mo, As, Cd, and Hg) increased significantly ( $P < 0.05$ ). These results suggest that

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Responsible editor: Philippe Garrigues						

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supplementation of high levels of arsenic affected trace mineral levels in the liver and kidney of chicken, and the effects vary from organ to organ. The aim of this study is to provide references for further study of heavy metal poisoning by detecting the contents of minerals induced by arsenic in chicken.

Keywords Chicken · Arsenic trioxide · Mineral levels · Liver . Kidney . ICP-MS

#### Introduction

Environmental pollution is now a major problem in developed, developing, and undeveloped countries (Liu et al. [2015](#page-9-0); Ahmed et al. [2015\)](#page-8-0). Arsenic is a toxic element that is also very common in the environments probably due to its wide application: smelting of metal ores, wood preservatives, lead-acid automobile batteries, semiconductors in telecommunications, pesticides, etc. (Adeyemi et al. [2015](#page-8-0); Coelho et al. [2012\)](#page-8-0). Metals from natural and anthropogenic sources, including arsenic, continuously enter the environment where they pose serious threat because of their toxicity, long persistence, bioaccumulation, and biomagnification in the food chain (Lynch et al. [2014](#page-9-0); Papagiannis et al. [2004\)](#page-9-0). In general, inorganic arsenic is the more toxic form than organic forms and is present in water, which is readily absorbed by the animal and human body (Shah et al. [2009](#page-9-0)). Chronic exposure to arsenic can lead to dermatitis, mild pigmentation keratosis of the skin, vasospasticity, gross pigmentation with hyperkeratinization of exposed areas, wart formation, decreased nerve conduction velocity, and lung cancer (Rodriguez et al. [2003;](#page-9-0) Ahmed et al. [2015\)](#page-8-0). For arsenic toxicity mechanism, recently, there have been many hypotheses such as the ability of arsenic to generate oxidative stress, induce apoptosis, chromosomal aberration, change the signaling model, and so on (Basu et al. [2001;](#page-8-0) Hei et al. [1998;](#page-8-0) Liu et al. [1996\)](#page-9-0). Although arsenic contamination in the environment has been reported worldwide (Li et al. [2011;](#page-8-0) Sohel et al. [2009\)](#page-9-0), drinking water contamination with arsenic has been a major public health concern in southeast, southwest, and northeast USA, inner Mongolia (China), southwest Taiwan coastal regions, Sonora (Mexico), Pamplonian Plain (Argentina), West Bengal (India), Northern Chile, and Bangladesh (Argos et al. [2010\)](#page-8-0).

Mineral elements have positive and negative effects on human health and the environment. Essential trace minerals are important for a wide variety of physiological processes in all animals. Several hundred enzymes require the presence of minerals for their activity (Yuan et al. [2011](#page-9-0)). An optimal level of mineral elements is important for the health of animals and humans. The ingestion of food is an obvious means of exposure to metals, not only because many metals are natural components of foodstuff but also because of the environmental contamination and contamination during processing (Harding et al. [2005](#page-8-0)). Toxic elements can be very harmful even at low concentration when ingested over a long period. The essential metals can also produce toxic effects when the metal intake is excessively elevated (Tuzen [2003;](#page-9-0) Celik and Oehlenschlager [2007](#page-8-0); Pouretedal and Rafat [2007\)](#page-9-0). Therefore, some studies are interested in the analysis of the trace metal contents of the environmental samples and especially food (Lin et al. [2013](#page-9-0); Uluozlu et al. [2009;](#page-9-0) Mondal et al. [2007\)](#page-9-0).

The effects of trace metal on birds may have diagnostic significance in the evaluation of adverse effects of trace metal to human health because birds have an important role in the food chain. Therefore, chicken is also selected as an experimental model for the prediction of the sensitivity of other avian species to the impact of environmental contaminants and the assessment of human health risk (Naraharisetti et al. [2009](#page-9-0); Nachman et al. [2012\)](#page-9-0). Many studies have described the adverse effects of arsenic on chicken (Sanchez-Virosta et al. [2015](#page-9-0); Shah et al. [2009](#page-9-0)). Liu et al. ([2012\)](#page-9-0) have reported effects of dietary manganese on Cu, Fe, Zn, Ca, Se, IL-1β, and IL-2 changes of immune organs in cocks. However, there is scarcity of information regarding the effects of arsenic on the mineral element contents of chicken tissue. Previous researches proved that various organs and tissues have various conditions in different concentrations of arsenic (Altikat et al. [2015\)](#page-8-0). Therefore, in the present study, we constructed as experiment serving male Hyline cocks an arsenic-supplemented diet and investigated the effects of arsenic-induced toxicity on the mineral element contents in the liver and kidney tissues of chicken.

## Materials and methods

# Animals and experimental design

All procedures used in the present study were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. A total of 100 male Hyline cocks (1 day old) were randomly divided into 2 groups ( $n = 50$ ), including an arsenic-treated group and a control group. The control group was fed with basal diet, and the arsenic-treated group was fed with the basic diet supplemented with arsenic toxicity  $(As<sub>2</sub>O<sub>3</sub>)$  at 30 mg/kg according to  $1/20$  of the median lethal dose  $(LD_{50})$  for cocks (Xing et al. [2015](#page-9-0)). Chickens were fed a basal diet with cereals, legumes, and their byproducts and without vitamins or microminerals. The composition of the diet was as follows: maize grains 421 g/kg, wheat grains 120 g/kg, full fat soy 180 g/kg, pea 100 g/kg, wheat bran 80 g/kg, limestone 80 g/kg, dicalcium phosphate 15 g/kg, and sodium chloride 4 g/kg. This diet met the minimum requirements for the energy and nutrients for the chickens and without influencing determination results according to Nisianakis et al. [\(2009\)](#page-9-0). Each group was separated into 6 pens (15 chickens per pen). Range areas were limited by metal fences and contained a food trough and a bell drinker. The feeding experiment lasted for 90 days and the experimental animals were given free access to feed and water. On day 90, the liver and kidney tissues were removed from individual chicks ( $n = 15$ ) after killing with sodium pentobarbital. The tissues were rinsed with icecold 0.9 % NaCl solution, frozen immediately in liquid nitrogen, and stored at −80 °C until required.

#### Mineral element analysis

The mineral elements lithium (Li), boron (B), sodium (Na), magnesium (Mg), aluminum (AL), silicon (Si), potassium (K), calcium (Ca), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo), cadmium (Cd), tin (Sn), antimony (Sb), barium (Ba), mercury (Hg), thallium (Tl), and lead (Pb) in the liver and kidney were determined using inductively coupled plasma mass spectrometry (ICP-MS) (Thermo iCAPQ, American). The instrumental parameters of the equipment used are summarized in Table [1](#page-2-0).

The mineral element concentrations were determined in the acid digest of the samples according to the method of Uluozlu et al. ([2009](#page-9-0)). One gram of each sample was digested with 5 mL HNO<sub>3</sub> (65 %) and 2 mL H<sub>2</sub>O<sub>2</sub> (30 %) in a microwave digestion system and diluted to 10 mL with deionized water. A blank digest was carried out in the same way. All sample solutions were clear. Digestion conditions for the microwave system were applied as 3 min for 1800 W at 100 °C, 10 min for 1800 W at 150 °C, and 45 min for 1800 W at 180 °C. The digested samples were filled with ultrapure water to the final volume before analysis by ICP-MS.

<span id="page-2-0"></span>Table 1 Instrumental parameters for the ICP-MS



#### Statistical analysis

Statistical analysis of all data was performed using SPSS for Windows (version 13, SPSS Inc., Chicago, IL). The differences between the arsenic-treated group and the control group were assessed by using paired  $t$  test. The data were expressed as the mean  $\pm$  standard deviation. Differences were considered to be significant at  $P < 0.05$ . In addition, principal component analysis (PCA) was used to define the most important parameters, which could be used as key factors for individual variations using Statistic 6.0.

# Results and discussion

Trace metals can be classified as potentially toxic (As, Cd, Pb, Hg, Sn, Al, Li), probably essential (V and Co), and essential (Cu, Zn, Fe, Mn, and Se) (Ebdon et al. [2001\)](#page-8-0). Arsenic contamination from natural and anthropogenic sources continuously enters the environment where they pose serious threat because of their toxicity, long persistence, bioaccumulation, and biomagnification in the food chain (Liu et al. [2015;](#page-9-0) Adeyemi et al. [2015](#page-8-0)). The main way of exposure to this metalloid occurs through drinking of contaminated water (Waghe

**Table 2** Effects of As on 26 element contents in the liver and kidney tissues of chicken  $(n = 5)$ 

Elements	Liver (ppb)		Kidney (ppb)	
	Control group	As-treated group	Control group	As-treated group
Li (STD)	$8.34 \pm 0.67$	$7.64 \pm 0.49$	$8.51 \pm 0.41$	$8.75 \pm 0.23$
B (KED)	$288.53 \pm 27.89$	$300.89 \pm 9.00$	$246.96 \pm 8.55$	$243.36 \pm 8.80$
Na (KED)	$1,102,768.6 \pm 63,017.9$	$1,129,088.9 \pm 72,383.6$	$1,585,710.0 \pm 93,621.4$	$1,757,571.9 \pm 62,645.8$
$Mg$ (KED)	$212,837.5 \pm 7087.7$	$199,832.6 \pm 11,031.9$	$211,972.8 \pm 3207.5$	$197,689.0 \pm 8706.3$
Al (KED)	$2140.42 \pm 151.35$	$1097.17 \pm 173.73*$	$1558.86 \pm 114.61$	$817.81 \pm 34.98*$
Si (STD)	$108,753.3 \pm 10,840.2$	$104,314.8 \pm 8861.2$	$97,148.7 \pm 3886.9$	$87,684.8 \pm 5197.2$
K(KED)	$2,907,835.9 \pm 298,253.5$	$2,899,321.2 \pm 110,116.1$	$2,786,246.6 \pm 67,598.9$	$2,474,329.9 \pm 45,838$ *
Ca (STD)	$69,810.18 \pm 7218.22$	$71,466.61 \pm 2343.13$	$137,218.1 \pm 5372.2$	$112,514.6 \pm 398.3^*$
$V$ (KED)	$49.50 \pm 3.33$	$48.38 \pm 4.82$	$37.11 \pm 1.56$	$39.35 \pm 2.25$
Cr(KED)	$145.16 \pm 8.80$	$145.95 \pm 12.24$	$257.97 \pm 16.58$	$94.06 \pm 7.42*$
Mn (KED)	$3278.53 \pm 174.87$	$2668.53 \pm 112.69*$	$3462.46 \pm 204.77$	$2645.34 \pm 79.70*$
Fe (KED)	$129,452.33 \pm 8648.97$	$120,651.83 \pm 10,636.83$	$64,659.3 \pm 2469.9$	$67,711.5 \pm 1843.3$
Co(STD)	$46.91 \pm 7.36$	$28.83 \pm 1.59*$	$48.07 \pm 2.89$	$44.43 \pm 2.79$
Ni (STD)	$34.00 \pm 7.61$	$74.29 \pm 5.33*$	$56.19 \pm 4.55$	$29.23 \pm 4.14*$
Cu (KED)	$4887.67 \pm 364.83$	$4201.12 \pm 161.26^*$	$3842.54 \pm 284.57$	$3847.68 \pm 320.57$
$Zn$ (KED)	$38,728.80 \pm 682.15$	$28,213.37 \pm 2579.19*$	$32,257.16 \pm 2058.09$	$29,132.56 \pm 2698.68$
As (STD)	$31.51 \pm 2.82$	$1074.83 \pm 65.22*$	$32.04 \pm 0.64$	$1638.97 \pm 92.44*$
Se (STD)	$852.16 \pm 24.27$	$598.86 \pm 43.49*$	$820.82 \pm 52.00$	$766.96 \pm 25.78$
Mo (STD)	$90.64 \pm 4.74$	$83.89 \pm 3.19$	$102.87 \pm 1.44$	$112.22 \pm 2.81*$
Cd (STD)	$50.26 \pm 3.99$	$25.83 \pm 4.64*$	$36.20 \pm 1.34$	$86.47 \pm 4.94*$
Sn (KED)	$14.38 \pm 0.52$	$13.64 \pm 0.60$	$12.53 \pm 0.71$	$12.87 \pm 0.39$
Sb (KED)	$17.09 \pm 0.81$	$17.08 \pm 0.68$	$18.20 \pm 1.19$	$15.10 \pm 0.49*$
Ba (KED)	$58.36 \pm 4.74$	$24.82 \pm 0.92^*$	$61.22 \pm 2.75$	$34.82 \pm 2.21*$
$Hg$ (KED)	$1.20 \pm 0.06$	$3.29 \pm 0.13*$	$1.02 \pm 0.17$	$6.35 \pm 0.37*$
Tl (KED)	$0.75 \pm 0.03$	$0.63 \pm 0.06$	$4.27 \pm 0.32$	$4.33 \pm 0.32$
Pb (KED)	$15.43 \pm 1.52$	$6.52 \pm 0.24*$	$26.78 \pm 2.62$	$20.14 \pm 0.69*$

The asterisk indicates that there are significant differences (\* $P < 0.05$ ) between the control group and As-treated group

<span id="page-3-0"></span>Fig. 1 Ordination diagram of principal component analysis (PCA) of parameters measured in the liver of chickens overexposed to  $As_2O_3$ 



et al. [2014\)](#page-9-0). Depending on the World Health Organization guidelines, arsenic in drinking water should not be beyond 0.01 ppm (WHO, [2010\)](#page-9-0). Chickens are a major source of meat for human consumption in North America and China. Chicken and eggs are the world's most consumed food. We put the layers as the research object, aiming to show the issues of chickens for meat and eggs in these two perspectives. With the characteristics of having a wide range of sources, low cost, and good meat quality, Hyline cocks were chosen for this study. In recent years, heavy metal poisoning has been a hot

Fig. 2 Ordination diagram of PCA of parameters measured in the kidney of chickens overexposed to  $As<sub>2</sub>O<sub>3</sub>$ 



<span id="page-4-0"></span>



area of research and many studies have described the adverse effects of heavy metal on chicken (Sun et al. [2011](#page-9-0); Liu et al. [2013;](#page-9-0) Yang et al. [2012\)](#page-9-0). Concentrations of arsenic in chickens range from μg/kg to mg/kg, and long-term arsenic exposure was linked to both carcinogenic and non-carcinogenic diseases. It was reported that arsenic toxicity can lead to oxidative damage, apoptosis, inflammatory response, and possibly even cancer (Xing et al. [2015;](#page-9-0) Guo et al. [2015](#page-8-0)). ICP-MS is well established as a method for multielemental analysis and the determination of isotope ratios (Guidotti et al. [2015;](#page-8-0) Aoun et al. [2015;](#page-8-0) Jovicic et al. [2015](#page-8-0)). Chevallier et al. ([2015](#page-8-0)) determined concentrations of 31 elements in foodstuff by ICP-MS. Giannenas et al. [\(2009\)](#page-8-0) reported the contents of the trace minerals Se, Zn, Mn, Co, Cu , Mo, V, Cr, Ni, Tl, As, and Cd in yolk and albumen from hen eggs using ICP-MS. This methodology allows simultaneous analysis of a wide range of mineral elements in the same sample and has been used in this study.

Results about the 26 element levels in the liver and kidney of chickens overexposed to  $As<sub>2</sub>O<sub>3</sub>$  $As<sub>2</sub>O<sub>3</sub>$  $As<sub>2</sub>O<sub>3</sub>$  are listed in Table 2. Results displayed that 12 element levels were significantly changed ( $P < 0.05$ ) in the liver of chickens exposed to  $\Delta s$ . Compared to the control group. The results also show  $As<sub>2</sub>O<sub>3</sub>$  compared to the control group. The results also show that nine element levels (Al, Mn, Co, Cu, Zn, Se, Cd, Ba, and Pb) were significantly decreased ( $P < 0.05$ ) in the chickens exposed to As 2 O <sup>3</sup> compared to the control chickens where three element levels (Ni, As, and Hg) increased significantly  $(P < 0.05)$ . In the kidney, 13 element levels were significantly changed ( $P < 0.05$ ) in chickens exposed to  $As_2O_3$  compared to the control group. On the other hand, nine element levels ( $\Lambda$ ) the control group. On the other hand, nine element levels (Al, K, Ca, Cr, Mn, Ni, Sb, Ba, and Pb) were significantly decreased ( $P < 0.05$ ) in the chickens exposed to As<sub>2</sub>O<sub>3</sub> compared to the control chickens where four element levels (Mo, As, Cd, and Hg) increased significantly ( $P < 0.05$ ). A proper amount of mineral is required for the normal function of all biochemical processes in the animal body. Xia et al. [\(2015](#page-9-0) ) reported that a combination of Mo and Cd leads to greater tissue damage and has a synergistic effect on kidney damage. Xu et al. ([2016](#page-9-0)) reported that the protective role of Se and toxic effect of Pb may be related to these changing ion profiles in chicken liver. Through ICP-MS, more elements can be determined accurately at the same time. Nisianakis et al. [\(2009\)](#page-9-0) has reported 12 elements (Se, Zn, Mn, Co, Cu, Mo, V, Cr, Ni, As, Cd, Tl) in different kinds of birds in both yolk and albumen by ICP-MS. Compared with the results of our control group (Se, Zn, Mn, Co, Cu, Mo, Cr, Ni, As, Cd, Tl), trace elements in yolk and albumen are lower than that in liver and kidney in chicken. Compared with the results of Se, Cd, Zn, and Cu levels reported by Pappas et al. [\(2011\)](#page-9-0) in 4- and 6 week-old broilers, Cd and Cu values were double in our study; Zn values were higher, while Se values were similar to the values reported by the previous study. Our result of Pd is similar to that reported by Gerber et al. [\(2009\)](#page-8-0). Mondal et al.

<span id="page-6-0"></span>



Table 4

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[\(2007\)](#page-9-0) reported that major mineral balance (Ca, P, and Mg) was not affected by the dietary Cu-salt and soybean oil supplementation both at days 21 and 42. These results are consistent with our results. Chronic exposure to high levels of the toxic metals can cause a variety of adverse health effects, including skin and internal cancers and cardiovascular and neurological effects (Li et al. [2013](#page-9-0); Rodriguez et al. [2003\)](#page-9-0). The obtained results indicate that after exposed to arsenic, trace metal levels were significantly influenced by arsenic supplementation. Hence, it is critical to balance the animal's requirement to maintain growth performance and the mineral levels in the diet. But, because of little research about the effects of toxic metals on mineral element contents in animals and humans, the effect of arsenic on mineral levels needs to be further studied, especially in birds.

PCA is a method that uses the ideas of dimension reduction to turn multiple indexes into fewer comprehensive indexes. Using PCA, all biochemical parameters measured in the present study were distinguished on the ordination plots that correspond to the first and second principle components (Figs. [1](#page-3-0) and [2\)](#page-3-0). The first two principal components in the liver and kidney took into account 77.151 % (PC1 = 54.445 %, PC2 = 22.706 %) and 79.614 % (PC1 = 63.684 %).  $PC2 = 12.930\%$ , respectively. Furthermore, the observed relationships among the parameters confirmed and quantified according to Spearman 's test (Tables [3](#page-4-0) and [4\)](#page-6-0) indicated that there was a good differentiation of the liver and kidney samples. PCA reveals that there were both positive and negative correlations between different ions in both liver and kidney. In liver, Mn, Zn, and Se had a high positive correlation with Ti but Fe and Na had a high negative correlation with Cr. In kidney, Mo had a high positive correlation with Hg, Cd, and As but a high negative correlation with Al, K, Ca, Cr, Mn, Ni, Sb, and Ba. By analyzing the rotated component matrix (Tables [3](#page-4-0) and [4](#page-6-0)), Mn, Zn, Cd, Ti, Se, Co, Ba, Al, Pb, and Cu showed a high correlation with component 1, while Cr, K, Si, and V showed a high correlation with component 2 in liver. In kidney, Ni, Sb, Ca, Si, Pb, Mg, Al, Cr, Ba, K, and Mn showed a high correlation with component 1, while Cu and B showed a high correlation with component 2. The ICP-MS is considered as the most prominent tool for the quantification of trace levels of a variety of elements owing to its multielement capacity, high analytical throughput, and low limit of detection (LOD). Millour et al. ([2011](#page-9-0)) reported that 21 elements were analyzed using ICP-MS in standard mode. Compared with his results, Mn, Co, Cu, Zn, Ni, Mo, and Cd is higher in our results. Chevallier et al. [\(2015\)](#page-8-0) reported that the use of microwave digestion in a closed vessel for sample preparation and the ICP-MS for detection provided an accurate determination of 31 trace and major elements in several types of food samples (matrices of animal or plant origin and infant food matrices). Most of his results are consistent with ours, but compared with his research, we have a higher result in Na,

<span id="page-8-0"></span>Mg, Al, K, Fe, Cu, and Zn. The differences between the trace metal contents of our results and the previous research could be due to many reasons. One could be the possible differences in terms of digestion and metabolism of the ingested feed by different species and subsequent deposition of metals that were already present in the diet. In addition, diet, location, and even the analytical methodology could be an explanation. Our studies indicated that the ICP-MS determination of the trace elements in the animals enables a rapid analysis with good precision and accuracy.

The interactions among ions have been shown to be involved in various physical and pathologic processes. Al-Waeli et al. (2012) reported that exposure to high concentrations of Cd increased the concentration of Cd, Cu, Sb, and V and decreased that of Se, Mn, and Fe, which revealed several correlations between essential. Absorbed Cd is preferably accumulated in the kidney and liver bound to MT. Xu et al. [\(2016\)](#page-9-0) has reported that Pb exposure decreased the Hg content and increased the Cd and Sn in chicken liver. Our study indicated that arsenic exposure also makes difference in the mineral element contents in chicken, and different elements vary from tissue to tissue. The mechanism underlying arsenic to trace elements needs further study.

#### Conclusion

The use of both microwave digestion in closed vessels for sample preparation and the ICP-MS for detection permitted an accurate determination of 26 elements in several types of food samples (liver and kidney of chicken). The obtained results indicate that supplementation of high levels of arsenic affected trace metal levels in the liver and kidney of chicken, and the effects vary from organ to organ. Thus, the results of the work suggest that it is important to monitor the concentration of the toxic metal arsenic in chickens for human health.

Acknowledgments This study was supported by the Natural Science Foundation of Heilongjiang Province (Grant No. C2015061).

#### Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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