



# Selenium Mitigates Cadmium-Induced Adverse Effects on Trace Elements and Amino Acids Profiles in Chicken Pectoral Muscles

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## Abstract

Cadmium (Cd), as one of the most toxic heavy metals, has become a widespread environmental contaminant and threatens the food quality and safety. The protective effect of selenium (Se) on Cd-induced tissue lesion and cytotoxicity in chicken has been extensively reported. The objective of this study was to investigate the antagonistic effect of Se on Cd-induced damage of chicken pectoral muscles via analyzing the trace elements and amino acids profiles. Firstly, 19 trace elements contents were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). The results showed that under Cd exposure, the contents of Cd, lead (Pb), mercury (Hg), aluminum (Al), and lithium (Li) were significantly elevated, and the contents of Se, iron (Fe), and chromium (Cr) were significantly reduced. However, supplementing Se significantly reversed the effects induced by Cd. Secondly, the amino acids contents were detected by L-8900 automatic amino acid analyzer. The results showed that supplementing Se increased significantly Cd-induced decrease of valine (Val), leucine (Leu), arginine (Arg), and proline (Pro). Thirdly, the results of principal component analysis (PCA) showed that cobalt (Co), manganese (Mn), silicium (Si), and Pro may play special roles in response to the process of Se antagonizes Cd-induced damage of pectoral muscles in chickens. In summary, these results indicated that different trace elements and amino acids possessed and exhibited distinct responses to suffer from Se and/or Cd in chicken pectoral muscles. Notably, Se alleviated Cd-induced adverse effects by regulating trace elements and amino acids profiles in chicken pectoral muscles.

**Keywords** Cadmium · Selenium · Trace element · Amino acid · Chicken pectoral muscle

## Introduction

Cadmium (Cd) is one of the most toxic and widely distributed heavy metals [21]. It is considered a global contaminant because of its extensive use in various industrial and agricultural

activities [36]. Cd can be found in a concentrated amount in plants and animals, consequently entering in the human body through the enriched food chains [3, 5]. Due to the non-biodegradability and durability of Cd, it is subjected to bioaccumulation, consequently harming human health [38]. Cd results in hypertension, renal damage, and liver disease in humans [45]. Thus, Cd intoxication is a serious problem for humans living in areas of environmental Cd pollution and areas exposed to food containing Cd residues [12, 15].

From the perspective of food quality and safety, Cd residues in meat products represent a danger to human health. In fact, recent studies have revealed the presence of Cd residue in muscles of cattle [8], sheep [25], and horse [19]. Chicken meat product is one of the components of the human diet with high nutritional value because it is rich in proteins and essential amino acids as well as it contains important micronutrients such as trace elements [2]. Although numerous studies demonstrated that Cd-induced hepatotoxicity [16], nephrotoxicity [33], and spleen damage [4] in chickens, few data are available regarding the mechanism of Cd toxicity in chicken muscles, particularly in the aspects of trace elements profiles and amino acid profiles.

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Selenium (Se) is an essential micronutrient in mammals since it plays important roles in cellular redox balance by acting as an antioxidant [10, 41, 42]. Thus, Se deficiency can lead to several diseases, such as liver damage [23], white muscle disease and disorder of ion profiles in chickens [43, 44], and keshan disease in humans [32]. Several studies showed that the beneficial effects of Se in alleviating Cd toxicity of livers [11, 27] and kidneys [6, 26] in rats. Moreover, other studies also showed that Se administration effectively mitigated the Cd-induced adverse effects on performance [1], antioxidant system [29], immunity system [40, 47], pancreatic tissue [14, 22], and kidney tissue [20] in chickens. As a consequence of that, it has been suggested that Se serves as an effective antagonist to Cd. However, the mechanism of Se in ameliorating Cd-induced adverse effects in chicken muscles is not yet clearly defined.

In the present study, mineral elements and amino acid profiles in chicken pectoral muscles were measured to investigate the adverse effects of Cd exposure and the ameliorating effects of Se. The data provided some new evidences for further toxicological studies following Cd exposure and new aspects in the mechanism used by Se in the detoxification of chicken muscles.

## Materials and Methods

### Birds and Experiment Design

All animals' management and treatment procedures used in the current study were approved by the Institutional Animal Care and Use Committee of Shandong Agricultural University. A total number of 128 Hy-Line Brown laying hens (31 weeks old) were randomly divided into four groups ( $n = 32$  per group). The chickens were maintained either on a basic diet (layer mixed feed, Heilongjiang Hefeng Animal Husbandry Co. Ltd., Harbin, China) (control group) containing 0.2 mg/kg Se (sodium selenite), a Se-supplemented diet (Se group) containing 2 mg/kg Se, a Cd-supplemented diet (Cd group) containing 150 mg/kg Cd (cadmium chloride), and a Se plus Cd diet (Se + Cd group) containing 2 mg/kg Se and 150 mg/kg Cd. Chickens had access to food and water ad libitum over the entire experimental period. After 90 days of feeding, ten chickens in each group were randomly selected, humanely killed, and breast muscles were collected and immediately stored at  $-80^{\circ}\text{C}$  until analyses.

### Trace Elements Contents Analysis

The contents of 12 essential microelements including Se, iron (Fe), zinc (Zn), copper (Cu), chromium (Cr), cobalt (Co), manganese (Mn), molybdenum (Mo), silicium (Si), nickel (Ni), boron (B), and vanadium (V), and seven potentially toxic

microelements including Cd, lead (Pb), mercury (Hg), arsenic (As), aluminum (Al), tin (Sn), and lithium (Li) in chicken pectoral muscles were measured using inductively coupled plasma mass spectrometry (ICP-MS) (ThermoCAPQ, USA). The elements concentration was determined in the acid digestion of the samples according to the protocol described by Uluzozlu et al. [37]. In detail, 1 g of each sample was digested using 5 mL  $\text{HNO}_3$  (65%) and 2 mL  $\text{H}_2\text{O}_2$  (30%) in a microwave digestion system and diluted to 10 mL with deionized water. A blank digestion was carried out in the same way. All the sample solutions became clear. The digestion conditions applied in the microwave system were the following: 3 min at 1800 W and at  $100^{\circ}\text{C}$ , 10 min at 1800 W and at  $150^{\circ}\text{C}$ , and 45 min at 1800 W and at  $180^{\circ}\text{C}$ . The digested samples were filled with ultrapure water to the final volume before analysis by ICP-MS.

### Free Amino Acids Contents Analysis

The amino acid analyzer (L-8900, HITACHI, Japan) was used according to the method described by Li et al. [17] to determine the contents of seven essential amino acids including threonine (Thr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), and lysine (Lys), and ten non-essential amino acids including aspartic acid (Asp), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), cysteine (Cys), tyrosine (Tyr), histidine (His), arginine (Arg), and proline (Pro) in chicken pectoral muscles.

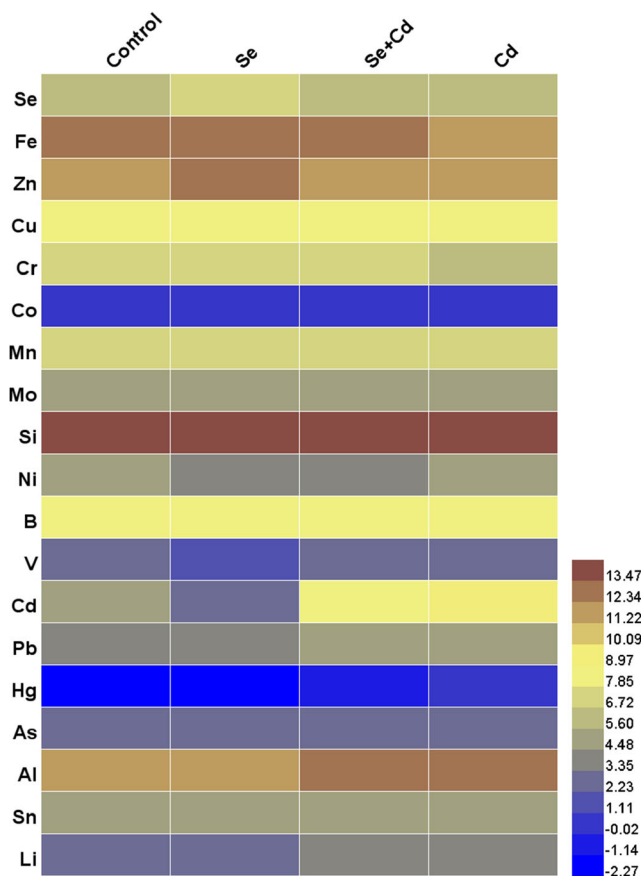
### Statistical Analysis

Statistical analysis was performed using Statistics 6.0 program (version 19, SPSS Inc., Chicago, USA). Unpaired or paired two-tailed  $t$  test was used for individual comparisons and one-way analysis of variance (ANOVA) followed by Duncan's test was used for multiple comparisons. Data were presented as the mean  $\pm$  standard deviation (SD) of at least three independent experiments. Differences were considered statistically significant at  $P < 0.05$ . Principal component analysis (PCA) was used to define the most important parameters using the Statistics 6.0 program. Heat maps were produced according to the methods described by Zheng et al. [48].

## Results

### Trace Elements Profiles

Nineteen trace elements including 12 essential microelements (Se, Fe, Zn, Cu, Cr, Co, Mn, Mo, Si, Ni, B, and V) and seven potentially toxic microelements (Cd, Pb, Hg, As, Al, Sn, and Li) were measured by ICP-MS in chicken pectoral muscles. As shown in Fig. 1, Cd treatment alone

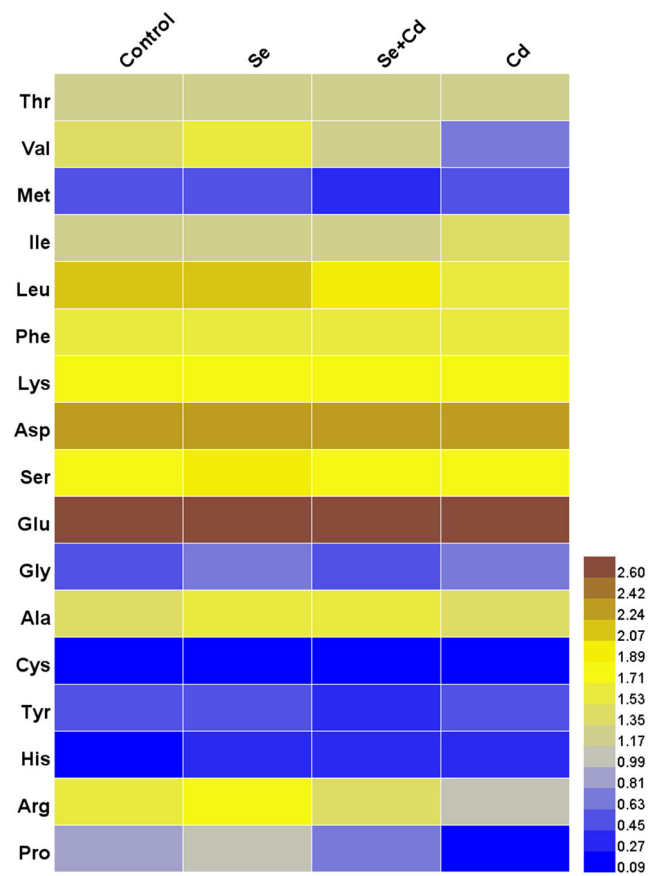


**Fig. 1** Heat map with intuitive reflection of the effects of Cd and Se on the mineral elements profiles in chicken pectoral muscles

decreased significantly the content of Se, Fe, and Cr ( $P < 0.05$ ). However, co-treatment with Se increased significantly their contents ( $P < 0.05$ ). The results also showed that Cd increased significantly the contents of Cd, Pb, Hg, Al, and Li ( $P < 0.05$ ); however, co-treatment with Se decreased significantly their contents ( $P < 0.05$ ). The results suggested that administration of Se and/or Cd primarily influenced the essential microelements and potentially toxic microelements in chicken pectoral muscles.

### Free Amino Acids Profiles

Seventeen free amino acid contents were measured in chicken pectoral muscles, including seven essential amino acids (Thr, Val, Met, Ile, Leu, Phe, and Lys), and ten non-essential amino acids (Asp, Ser, Glu, Gly, Ala, Cys, Tyr, His, Arg, and Pro). As shown in Fig. 2, a significant decrease of Val, Leu, Arg, and Pro was obtained in Cd group ( $P < 0.05$ ). However, Cd-induced their decrease was significantly restored by Se treatment ( $P < 0.05$ ). These results suggested that Se and/or Cd treatment could affect both essential amino acids and non-essential amino acids in chicken pectoral muscles.

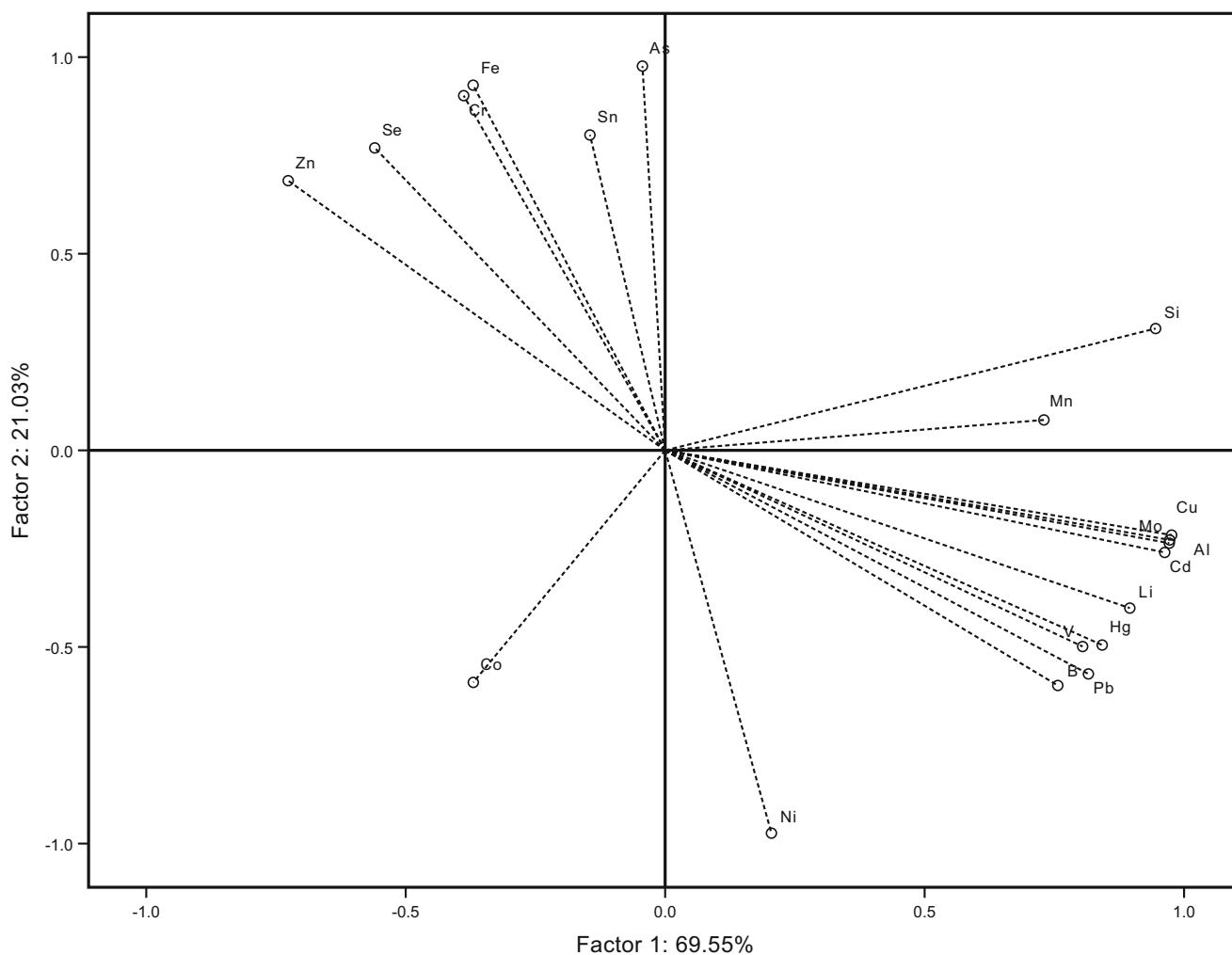


**Fig. 2** Heat map with intuitive reflection of the effects of Cd and Se on the amino acid profiles in chicken pectoral muscles

### Principal Component Analysis

We used PCA to define the most important parameters, which could be used as key factors for individual variations. As shown in Fig. 3, all parameters were divided into the first and second principle components (69.55 and 21.03%) based on ordination plots corresponding. In addition, predominantly trace elements had a strong positive correlation with component one, but Se, Fe, Zn, Cr, Co, As, and Sn were negatively correlated with component one. Trace elements had a relatively strong positive correlation with component two, but Cu, Co, Mo, Ni, B, V, Cd, Pb, Hg, Al, and Li had a relatively strong negative correlation with component two. The results indicated that Co, Mn, and Si may play special roles in response to Se and/or Cd exposure (Table 1).

As shown in Fig. 4, all parameters were divided into the first and second principle components (44.68 and 37.77%) based on ordination plots corresponding. Amino acids have a strong positive correlation with component one, but Ile, Lys, ASP, Glu, Cys, and Tyr are negatively correlated with component one. Predominantly, amino acids had a relatively strong positive correlation with component two, but Leu had a relatively strong negative correlation with component two. The



**Fig. 3** Principal component analysis for trace elements profiles. The rotating components in space. Ordination plots corresponding to the first and second principle components were 69.55 and 21.03%

results indicated that Pro may play special roles in response to administration of Se and/or Cd (Table 2).

**Discussion**

Cd is an environmental pollutant, can threaten greatly food quality and safety, and enter in the human body via the food chains. Many studies were performed to study Cd toxicity mechanisms and the antagonistic mechanisms of Se to Cd toxicity in chickens. However, little attention has been given to the role of Cd and Se in chicken muscles. In the present

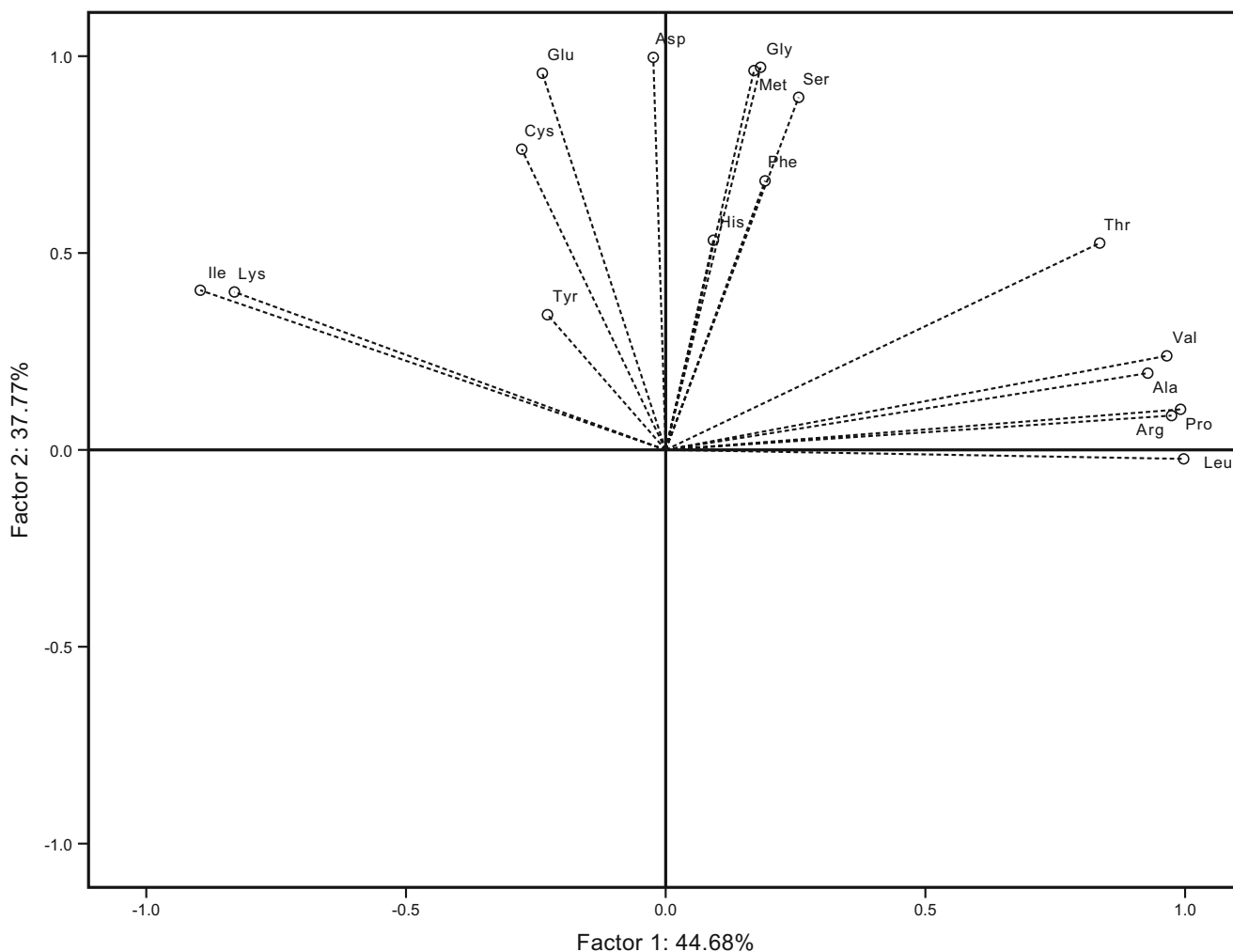
study, we examined the effect of Cd and Se on trace elements and amino acid profiles in chicken pectoral muscles.

Homeostasis of trace elements plays important roles in physiological activities including the maintenance of protein structures and catalysis of enzymatic reactions in humans and animals. Thus, their deficiency or excess leads to nutritional and metabolic diseases and toxonosis [35]. On one hand, type 2 diabetes and cardiovascular disease in humans are associated with Fe and Zn disorders [18]. On the other hand, toxic elements (Cd, Pb, Hg, As, Al, etc.) accumulation results in multi-organ and multiple systems diseases, such as immune system disease, nervous system disease, and reproductive

**Table 1** Rotating component matrix for trace elements profiles

Component	Se	Fe	Zn	Cu	Cr	Co	Mn	Mo	Si	Ni	B	V	Cd	Pb	Hg	As	Al	Sn	Li
1	-0.56	-0.37	-0.73	0.97	-0.39	-0.37	0.73	0.97	0.95	0.20	0.76	0.80	0.96	0.82	0.84	-0.04	0.98	-0.14	0.90
2	0.77	0.93	0.69	-0.24	0.90	-0.59	0.08	-0.23	0.31	-0.97	-0.60	-0.50	-0.26	-0.57	-0.50	0.98	-0.22	0.80	-0.40

Rotating convergence after three iteration



**Fig. 4** Principal component analysis for amino acids profiles. The rotating components in space. Ordination plots corresponding to the first and second principle components were 44.68 and 37.77%

system impairment [24]. Several studies revealed that Cd exposure can affect the balance of mineral elements in animals [7] and humans [9]. In addition, the content of trace elements is closely related to the meat quality. It is therefore of great importance to monitor the levels of trace elements in the food supply, including chicken meat. In this study, 12 essential microelements and 7 potentially toxic microelements were measured using ICP-MS. The results showed that the decrease of Se, Cr, and Fe and the increase of Li, Al, Cd, Hg, and Pb induced by Cd exposure were noticeably alleviated by Se treatment. These results were consistent with previous reports, which concluded that Se is negatively correlated with Cd and

positively correlated with Fe [29]. Zhang et al. and Jin et al. reported that Se ameliorated significantly Cd-induced elevation of the contents of Co, Mo, and Pb in chicken kidneys [46], and Li, Al, thallium (Tl), and Pb in chicken pancreas [14]. Combined with our results, it is indicated that different tissue possesses and exhibits distinct responses to suffer from Se and/or Cd. Interestingly, different heavy metals, such as Pb and As, in chicken muscles possess and exhibit distinct influences to trace elements profiles [13, 34].

Chicken meat is preferred by consumers because of its low-fat content and high concentration of polyunsaturated fatty acids, essential amino acids, and other bioactive substances,

**Table 2** Rotating component matrix for amino acids profiles

Component	Thr	Val	Met	Ile	Leu	Phe	Lys	Asp	Ser	Glu	Gly	Ala	Cys	Tyr	His	Arg	Pro
1	0.84	0.96	0.17	-0.90	1.00	0.19	-0.83	-0.02	0.26	-0.24	0.18	0.93	-0.28	-0.23	0.09	0.97	0.99
2	0.53	0.24	0.96	0.41	-0.02	0.68	0.40	1.00	0.90	0.96	0.97	0.19	0.76	0.34	0.53	0.09	0.10

Rotating convergence after three iteration



which are beneficial to humans' health [30]. The biological value of proteins in muscles is anchored in their amino acids composition, including Lys, His, Arg, Leu, Ile, and Val. Prior studies indicated that essential amino acids content is an important factor in determining the nutritional value of meat [31], and the composition of free amino acids is indispensable to the meat taste properties [28]. Thus the content of free amino acids in meat products is a crucial parameter, which is used to establish their quality. In the present study, our results showed that Cd-induced essential amino acids disorder involving in Val and Leu, and no essential amino acids disorder involving in Arg and Pro. These results were in agreement with previous studies, which revealed that the amino acids profiles in chicken muscles were susceptible to heavy metals and other elements [17, 39].

Collectively, the results of this study showed that Cd exposure affected the contents of trace elements and amino acids in chicken pectoral muscles, and Se could mitigate the adverse effects caused by Cd exposure. This study provided references for further study of the toxicological mechanism of Cd and may be helpful to understanding the mechanism of Se in antagonizing muscular toxicity in chickens induced by Cd.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Abbreviations** Se, Selenium; Fe, Iron; Zn, Zinc; Cu, Copper; Cr, Chromium; Co, Cobalt; Mn, Manganese; Mo, Molybdenum; Si, Silicon; Ni, Nickel; B, Boron; V, Vanadium; Cd, Cadmium; Pb, Lead; Hg, Mercury; As, Arsenic; Al, Aluminum; Sn, Tin; Li, Lithium; Sb, Stibium; Tl, Thallium; ICP-MS, Inductively coupled plasma mass spectrometry; Thr, Threonine; Val, Valine; Met, Methionine; Ile, Isoleucine; Leu, Leucine; Phe, Phenylalanine; Lys, Lysine; Asp, Aspartic acid; Ser, Serine; Glu, Glutamic acid; Gly, Glycine; Ala, Alanine; Cys, Cysteine; Tyr, Tyrosine; His, Histidine; Arg, Arginine; Pro, Proline

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