Assessment of Cadmium, Chromium, and Lead Contamination Levels in Compound Feed for Laying Hens in Sichuan, Shandong, and Hebei Provinces, China: A Postprint

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Abstract

This experiment aimed to determine the contents of cadmium (Cd), chromium (Cr), and lead (Pb) in compound feed for layer chickens during peak laying period from farms in Sichuan, Shandong, and Hebei provinces (including the Beijing-Tianjin-Hebei region) of China, and to assess the contamination levels of these three heavy metals in major layer chicken farming provinces. A total of 268 compound feed samples for layer chickens were collected from 67 farms in Sichuan, Shandong, and Hebei provinces, and their Cd, Cr, and Pb contents were determined using high-resolution continuum source flame atomic absorption spectrometry (HR-CS FAAS). The results showed: 1) In Sichuan province, the cities with the maximum detected values of Cd, Cr, and Pb in layer chicken compound feed were Chengdu, Leshan, and Leshan, respectively, with maximum detected values of 0.48, 15.94, and 14.12 mg/kg; the cities with the highest exceedance rates for Cr and Pb were Leshan and Chongzhou, with exceedance rates of 30% and 50%, respectively. 2) In Shandong province, the cities with the maximum detected values of Cd, Cr, and Pb in layer chicken compound feed were Qingdao and Rizhao, Heze, and Rizhao, respectively, with maximum detected values of 0.45, 5.88, and 4.11 mg/kg. 3) In Hebei province, the cities with the maximum detected values of Cd, Cr, and Pb in layer chicken compound feed were Zhangjiakou, Zhangjiakou, and Baoding, respectively, with maximum detected values of 0.45, 15.38, and 8.38 mg/kg; the cities with the highest exceedance rates for Cr and Pb were Zhangjiakou and Baoding, with exceedance rates of 20% each. 4) The average contents of Cd, Cr, and Pb in layer chicken compound feed from the three provinces were as follows: Sichuan: 0.24, 5.01, 3.67 mg/kg; Shandong: 0.26, 4.19, 2.61 mg/kg; Hebei: 0.25, 5.66, 3.25 mg/kg. According to China' s "Hygienical Standard for Feeds" , the Cd

content in layer chicken compound feed from the three provinces and the Cd, Cr, and Pb contents in Shandong province did not exceed the standard limits; the exceedance rates for Cr and Pb in Sichuan and Hebei provinces were 9.38% and 17.19%, and 6.73% and 14.42%, respectively. 5) The average contents of Cr and Pb in layer chicken compound feed from Hebei province were significantly higher than those from Shandong province ($P < 0.05$); the average contents of Cd, Cr, and Pb in compound feed from large-scale farms were significantly lower than those from small-scale farms ($P < 0.05$). In summary, the contents of Cd, Cr, and Pb in layer chicken compound feed from Shandong province did not exceed the standard limits. The Cd content in layer chicken compound feed from Sichuan and Hebei provinces did not exceed the standard limits but posed a risk of exceedance; the Cr and Pb contents exceeded the standard limits to varying degrees, with Pb exceedance being the most severe, which warrants attention.

Full Text

Contamination Assessment of Cadmium, Chromium, and Lead in Compound Feed for Laying Hens in Sichuan, Shandong, and Hebei Provinces, China

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Abstract: This study aimed to determine the contents of cadmium (Cd), chromium (Cr), and lead (Pb) in compound feed for laying hens during peak production in major poultry-producing provinces of Sichuan, Shandong, and Hebei (including the Beijing-Tianjin-Hebei region), and to assess the contamination levels of these three heavy metals. A total of 268 feed samples were collected from 67 laying hen farms across the three provinces and analyzed using high-resolution continuum source flame atomic absorption spectrometry (HR-CS FAAS). The results showed: 1) In Sichuan, the maximum detected values of Cd, Cr, and Pb were 0.48 mg/kg (Chengdu), 15.94 mg/kg (Leshan), and 14.12 mg/kg (Leshan), respectively. The highest over-limit rates for Cr and Pb occurred in Leshan (30%) and Chongzhou (50%), respectively. 2) In Shandong, the maximum detected values were 0.45 mg/kg (Qingdao and Rizhao), 5.88 mg/kg (Heze), and 4.11 mg/kg (Rizhao) for Cd, Cr, and Pb, respectively. 3) In Hebei, the maximum detected values were 0.45 mg/kg (Zhangjiakou), 15.38 mg/kg (Zhangjiakou), and 8.38 mg/kg (Baoding) for Cd, Cr, and Pb, respectively. The highest over-limit rates for Cr and Pb were both 20%, occurring in

Zhangjiakou and Baoding. 4) The average contents across the three provinces were: Sichuan (0.24, 5.01, and 3.67 mg/kg), Shandong (0.26, 4.19, and 2.61 mg/kg), and Hebei (0.25, 5.66, and 3.25 mg/kg) for Cd, Cr, and Pb, respectively. According to China' s National Hygienic Standard for Feeds, Cd levels in all provinces and all metals in Shandong were within limits, while Cr and Pb in Sichuan and Hebei exceeded standards, with over-limit rates of 9.38% and 17.19% in Sichuan, and 6.73% and 14.42% in Hebei, respectively. 5) Average Cr and Pb contents in Hebei were significantly higher than in Shandong ($P < 0.05$), and large-scale farms showed significantly lower Cd, Cr, and Pb levels than small-scale farms ($P < 0.05$). In conclusion, Shandong' s feed showed no over-limit contamination. While Cd in Sichuan and Hebei remained within limits, it approached threshold values, indicating potential risk. Cr and Pb contamination in these two provinces exceeded standards to varying degrees, with Pb being the most serious concern requiring immediate attention.

Keywords: HR-CS FAAS; compound feed for laying hens; heavy metals; cadmium; chromium; lead

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As a highly nutritious livestock product, eggs are produced and consumed in large quantities in China. However, food safety incidents involving eggs have occurred frequently, such as the Sudan Red incident in 2005, the toxic egg incident in the United States in 2013, and the counterfeit egg incident in Germany in 2014. Cadmium (Cd), chromium (Cr), and lead (Pb) are three highly toxic heavy metals. In recent years, rapid industrial and agricultural development has caused severe contamination of air, soil, and water with these metals, which subsequently accumulate in crops and contaminate feed ingredients. Additionally, some manufacturers use low-quality mineral supplements to reduce costs, further contributing to heavy metal exceedances in compound feed [1-2]. Feed contamination represents the primary pathway for heavy metal accumulation in eggs, and these metals pose severe health risks to both animals and humans. Once contaminated, heavy metals in feed and eggs are difficult to eliminate. Therefore, assessing Cd, Cr, and Pb contamination in laying hen feed in major poultry-producing provinces (Sichuan, Shandong, and Hebei) is crucial for evaluating egg safety and feed quality in these regions.

Detection technologies for Cd, Cr, and Pb include traditional line-source atomic absorption spectrometry (AAS) [3-4], inductively coupled plasma mass spectrometry (ICP-MS) [5-6], and high-resolution continuum source atomic absorption spectrometry (HR-CS FAAS) [7-9]. Traditional AAS cannot simultaneously determine multiple elements and is now less commonly used, while ICP-MS remains expensive and difficult to popularize. HR-CS FAAS has become widely applied due to its multi-element capability and relatively low instrument cost. Meng et al. [8] used wet digestion-flame AAS to determine trace elements in feed and eggs from a poultry farm in Henan, finding that both feed and corresponding eggs exceeded National Hygienic Standard limits for Pb and Cd. Yuan et al. [5] used ICP-MS to analyze 13 heavy metals in feed and eggs from Sichuan,

reporting Cd levels within the 0.5 mg/kg limit but Cr and Pb exceedances (10 and 5 mg/kg limits) at rates of 11.1% and 18.2%, respectively. Tu et al. [10] analyzed heavy metals in 96 aquafeed samples from Fujian, finding Pb levels of 0.05–3.52 mg/kg (within the 5 µg/g safety limit) but Cd and Cr exceedances in some samples (0.08–3.05 mg/kg and 1.28–20.26 mg/kg, respectively), with severe Cr contamination. Wang et al. [11] investigated heavy metals in livestock feed in North China, revealing significant variation among farms, with pig and beef cattle feed showing the most severe contamination, followed by broiler and laying hen feed. While all samples were within Cd limits, Cr and Pb exceedances were common, with laying hen feed showing a 53.85% Pb exceedance rate. Zhu et al. [12] analyzed heavy metals in fattening pig feed from 64 large-scale farms in Shaanxi, finding all three metals exceeded national standards, with maximum exceedance multiples of 110.86, 5.44, and 7.67 for Cd, Cr, and Pb, respectively.

In summary, feed heavy metal contamination is severe and shows an increasing accumulation trend in poultry eggs [13]. Sichuan, Shandong, and Hebei are major livestock provinces with developed modern industries and serious environmental pollution that threatens animal health. However, few reports have mapped the regional distribution of these three heavy metal risk factors across these provinces. Therefore, this study employed HR-CS FAAS to simultaneously determine Cd, Cr, and Pb contents in laying hen feed from farms in these provinces, assess contamination levels, and provide data for feed and egg safety evaluation.

1.1 Sample Collection

Compound feed samples were collected from laying hens during peak production between January and August 2016 from 67 farms of different scales in Sichuan, Shandong, and Hebei provinces (including Beijing-Tianjin-Hebei region, hereinafter collectively referred to as Hebei). Four parallel samples (500 g each) were collected per farm, totaling 268 samples. Following the Feed Sampling standard (GB/T 14699.1-2005) [14], samples comprised 64 feed samples from 16 farms across one provincial capital and six prefecture-level cities in Sichuan (covering eastern, western, southern, and northern regions); 100 samples from 25 farms across one provincial capital and 24 prefecture-level cities in Shandong; and 104 samples from 26 farms across four prefecture-level cities and two surrounding areas (Jixian County in Tianjin and Daxing and Yanqing districts in Beijing) in Hebei. All samples were stored at -20°C until analysis. The sampling distribution is shown in Table 1 .

1.2 Analytical Methods

Cd, Cr, and Pb contents in compound feed were determined by HR-CS FAAS as follows: feed samples were ground through a 40-mesh sieve, and 0.5 g was accurately weighed into a polytetrafluoroethylene digestion vessel. After adding 8 mL nitric acid (HNO) and 2 mL hydrogen peroxide, samples were pre-digested in a constant temperature heater for 30 minutes, then digested in a microwave

digestion system, and finally diluted to 25 mL with 0.5% (v/v) HNO solution. HR-CS FAAS working conditions are shown in Table 2 .

1.3 Instruments and Reagents

Main instruments: ContrAA-700 high-resolution continuum source atomic absorption spectrometer (equipped with MPE-60 autosampler, Analytik Jena, Germany); CEM-MARS6 intelligent microwave digestion system (USA); BHW-09Y constant temperature heater (Shanghai Botong Chemical Technology Co., Ltd.).

Main reagents: HNO and 30% (V/V) hydrogen peroxide (both analytical grade, Guangdong Xilong Chemical); Cd, Cr, and Pb standard solutions (1,000 μg/mL) from the National Standard Material Center, diluted to required concentrations; ultrapure water (18.2 MΩ • cm) from a Millipore system (USA).

1.4 Statistical Analysis

Data were analyzed using SPSS 19.0 software. Duncan' s multiple range test was used to compare differences in feed heavy metal contents among regions (Sichuan, Shandong, Hebei) and farm scales, with $P < 0.05$ as the significance threshold. Results are expressed as mean ± standard deviation.

2.1 Analytical Speed, Accuracy, and Detection Limits of HR-CS FAAS

As shown in Table 3 , HR-CS FAAS simultaneously determined Cd, Cr, and Pb in one feed sample within 2 hours, with recoveries of 96.0–101.0%, 98.6–99.9%, and 95.4–101.3%, respectively. Detection limits (LOD) were 1.7, 19.7, and 34.6 ng/mL for Cd, Cr, and Pb. Thus, HR-CS FAAS provides accurate and rapid determination suitable for analyzing these metals in laying hen feed.

2.2.1 Distribution of Cd, Cr, and Pb in Sichuan Province

As shown in Tables 4 , 5 , and 6 , Cd levels in 64 feed samples from seven Sichuan cities did not exceed limits, with the maximum value (0.48 mg/kg) found in Chengdu. Maximum Cd values in Chengdu, Deyang, Leshan, and Ya' an approached the National Hygienic Standard limit (0.5 mg/kg), indicating potential exceedance risk. While Cr and Pb levels in Chengdu, Deyang, Mianyang, Nanchong, and Ya' an were within limits, both metals exceeded standards in Leshan (Cr: 30% over-limit rate, mean 13.45 mg/kg, max 15.94 mg/kg; Pb: 45% over-limit rate, mean 8.67 mg/kg, max 14.12 mg/kg). Pb also exceeded limits in Chongzhou (50% over-limit rate, mean 5.59 mg/kg). Overall, maximum Cd, Cr, and Pb values occurred in Chengdu, Leshan, and Leshan, respectively, while the highest Cr and Pb over-limit rates were in Leshan and Chongzhou.

2.2.1 Distribution of Cd, Cr, and Pb in Shandong Province

As shown in Tables 7 , 8 , and 9 , all 100 feed samples from nine Shandong cities were within National Hygienic Standard limits, indicating relatively light contamination. Maximum Cd values were 0.45 mg/kg in Qingdao and Rizhao, with highest mean values in Yantai (0.31 mg/kg) and Rizhao (0.34 mg/kg). Maximum Cr values were 6.17 mg/kg (Heze) and 5.88 mg/kg (Dezhou), with highest means in Heze (5.42 mg/kg) and Liaocheng (4.95 mg/kg). Maximum Pb values were 3.88 mg/kg (Yantai) and 4.11 mg/kg (Rizhao), with highest means in Yantai (3.14 mg/kg) and Linyi (3.03 mg/kg).

2.2.1 Distribution of Cd, Cr, and Pb in Hebei Province

As shown in Tables 10 , 11 , and 12 , Cd levels in 104 feed samples from six Hebei cities did not exceed the 0.5 mg/kg limit, with maximum values of 0.45 mg/kg in Zhangjiakou and 0.44 mg/kg in Baoding. However, Cr and Pb exceeded limits in Zhangjiakou and Baoding. Cr maximum values were 18.35 mg/kg (Zhangjiakou) and 15.38 mg/kg (Baoding), both exceeding the 10 mg/kg limit with over-limit rates of 20.00% and 7.50%, respectively (mean exceedance values: 13.45 and 11.32 mg/kg). Pb maximum values were 8.38 mg/kg (Baoding) and 7.32 mg/kg (Zhangjiakou), exceeding the 5 mg/kg limit with over-limit rates of 20.00%, 12.50%, and 15.00% across cities (mean exceedance values: 7.54, 6.81, and 6.43 mg/kg). Zhangjiakou showed the highest Cr and Pb over-limit rates and most severe contamination, followed by Baoding.

2.3 Comparison of Cd, Cr, and Pb Contamination Among Three Provinces

As shown in Table 13 , Cd contents did not differ significantly among provinces ($P > 0.05$), with average levels ranking Shandong > Hebei > Sichuan, all within the 0.5 mg/kg limit. Cr and Pb exceeded limits in Sichuan and Hebei, with over-limit rates of 9.38% and 17.19% in Sichuan, and 6.73% and 14.42% in Hebei (mean exceedance values: 13.45 and 8.11 mg/kg in Sichuan; 15.55 and 6.93 mg/kg in Hebei). Cr content in Sichuan did not differ significantly from Shandong or Hebei ($P > 0.05$), but Hebei's average was significantly higher than Shandong' s ($P < 0.05$). Pb averages in Sichuan and Hebei were significantly higher than in Shandong ($P < 0.05$).

2.4 Contamination in Different Farm Scales

Based on farm size ranges, farms were categorized as small (<10,000 birds), medium-small (10,000–20,000), medium (20,000–50,000), and large (>100,000). As shown in Table 14 , metal contents varied significantly by scale. Cr and Pb exceeded limits in small, medium-small, and medium-scale farms, but not in large-scale farms. Cd and Cr contents generally decreased with increasing farm size (small > medium-small > medium > large), with small-scale farms significantly higher than large-scale ($P < 0.05$). Pb content was highest in

medium-scale and lowest in large-scale farms, with medium, small, and medium-small farms significantly higher than large-scale ($P < 0.05$). Pb showed the highest over-limit rates and most severe contamination, particularly in medium-small and small-scale farms.

3.1 Contamination Levels and Distribution in Laying Hen Feed

Contamination levels varied significantly by region and farm scale. In Sichuan, severe Cr and Pb exceedances occurred in Leshan and Chongzhou, primarily from small and medium-small farms with complex ingredient sources and crude processing/transportation technologies. One farm' s Pb content exceeded the U.S. Mineral Tolerance of Animals limit (10 mg/kg), likely due to low-quality ingredients and unscientific self-formulation practices. In contrast, industrially developed Chengdu, Deyang, and Mianyang showed no exceedances, while Ya' an with less heavy industry had low metal levels. Overall, Sichuan' s over-limit rates were 0% for Cd, 9.38% for Cr, and 17.19% for Pb, consistent with Yuan et al. [5] for Cd and Cr but higher for Pb, possibly due to sample size differences and sources (Yuan' s samples were from large feed manufacturers, while ours were mainly from small-medium individual farms).

Shandong showed 100% detection rates but no exceedances for any metal, indicating lighter contamination than Sichuan and Hebei. Despite predominantly small-medium scale sampling, all metals were within limits, likely reflecting Shandong' s feed industry characteristics: shrinking self-formulation market, elimination of ~20% of small/obsolete enterprises, expansion of large companies, and improved small-medium enterprise performance due to low raw material prices and recovering farming conditions. Additionally, Shandong' s environmental reports show pollution mainly from atmospheric and water sources (particulate matter and sulfur dioxide) rather than heavy metals [16].

In Hebei, Cr and Pb exceedances were concentrated in Zhangjiakou and Baoding, possibly due to intensive livestock operations contaminating soil and water through manure, creating a vicious cycle [17], and because these are key chemical industry areas with environmental pollution [18-19]. Beijing samples showed no exceedances, likely because they came from large-scale farms in ecological conservation and new urban development zones. Wang et al. [11] reported higher exceedance rates for laying hen feed in North China (including Shandong and Hebei) at 0% for Cd, 34.86% for Cr, and 53.85% for Pb, possibly due to broader regional coverage, complex sample sources, inclusion of polluted areas, and strengthened regulatory oversight in recent years.

Comprehensively, Shandong showed no exceedances and light contamination, while Sichuan and Hebei had varying Cr and Pb exceedances significantly higher than Shandong. Cd remained within limits across all provinces but approached threshold values, posing potential risk. Cr and Pb exceedances in Sichuan and Hebei may relate to samples mainly from small-medium farms and high environmental metal levels in soil and water [18-20].

Farm scale analysis revealed decreasing metal contents from small to large farms, with large-scale farms significantly lower than small-scale farms. Small and medium-small farms lack scientific, scaled ingredient sourcing, causing severe heavy metal contamination from the source. Excessive mineral additive use in self-formulated feed from small-medium farms also contributes to contamination. Additionally, improper use of machinery and utensils during processing, transportation, and storage represents another contamination source.

3.2 Potential Pollution Sources of Cd, Cr, and Pb in Feed

Heavy metal contamination sources in laying hen feed are widespread, primarily from animal/plant feed ingredients and mineral additives. Animal-derived ingredients including bone meal, leather protein powder, and fish meal contain excessive Cd, Cr, and Pb, particularly adulterated fish meal [21-22]. Plant-derived ingredients such as corn, corn by-products, wheat, distillers dried grains with solubles (DDGS), and cottonseed meal have shown varying metal exceedances, with Cr and Pb being particularly severe in some ingredients like corn and wheat bran [23-24]. Excessive mineral additive use also causes contamination [25]; for example, feed-grade phosphates are the main Cr source, with defluorinated phosphate and monocalcium phosphate averaging 110 and 83 mg/kg Cr [1], while feed-grade copper sulfate can contain 640 mg/kg Pb [2]. Modern industrial/agricultural development, especially uncontrolled emissions from mining and smelting areas [26-27] and pesticide/fertilizer misuse [27-28], further contaminates feed ingredients. Additionally, improper machinery and utensil use during processing contributes to contamination [28].

3.3 Hazards of Cd, Cr, and Pb Contamination in Feed

Cd, Cr, and Pb exhibit multi-system and multi-organ toxicity with carcinogenic, teratogenic, and mutagenic effects [29]. Cd causes chronic poisoning through slow accumulation, primarily damaging renal tubular reabsorption and causing proteinuria, aminoaciduria, and glucosuria, while reducing laying performance, immunity, and egg quality [29-31]. Cr exists as Cr^3 and Cr in animals; Cr^3 assists insulin and is essential for glucose and cholesterol metabolism, but Cr is toxic, affecting redox processes, precipitating nucleic acids, and disrupting enzyme systems [32]. Cao [33] found that low Cr doses (<10 mg/kg) promoted egg production and improved quality (shell thickness and strength), while high doses (>600 mg/kg) significantly reduced hatchability and damaged liver and kidneys. Pb poisoning primarily damages hematopoietic organs and kidneys, with additional effects on respiration, immunity, and embryonic development [34]. As a non-essential element with strong accumulation and toxicity, high Pb doses (100 mg/kg) reduce body weight, carcass weight, antioxidant capacity, and egg quality, affecting fatty acid formation in liver and serum [35-36]. Jeng et al. [37] found that laying ducks consuming water containing 1.3 mg/kg Cd and 6.7 mg/kg Pb showed significantly reduced body weight, egg production, and egg weight. Human consumption of eggs with accumulated heavy metals

can cause developmental delays, hearing loss, and kidney and cardiovascular damage in infants and adolescents [38-39].

This study found that although Cd levels were within limits, their proximity to threshold values poses serious long-term risks. Cr and Pb exceedances in Sichuan and Hebei (over-limit rates: Sichuan 9.38% and 17.19%; Hebei 6.73% and 14.42%) and significantly higher levels in small-scale versus large-scale farms demand urgent attention from feed manufacturers and regulators. Regular monitoring of Cd, Cr, and Pb in feed and eggs is imperative. Given the complexity of ingredient sources and potential batch-to-batch variation, further analysis of different feed batches is needed to clarify dynamic contamination patterns and distribution across these provinces.

Conclusions:

1) In Sichuan, maximum Cd, Cr, and Pb values were 0.48 mg/kg (Chengdu), 15.94 mg/kg (Leshan), and 14.12 mg/kg (Leshan), respectively, with highest Cr and Pb over-limit rates in Leshan (30%) and Chongzhou (50%). In Shandong, maximum values were 0.45 mg/kg (Qingdao and Rizhao), 5.88 mg/kg (Heze), and 4.11 mg/kg (Rizhao). In Hebei, maximum values were 0.45 mg/kg (Zhangjiakou), 15.38 mg/kg (Zhangjiakou), and 8.38 mg/kg (Baoding), with highest Cr and Pb over-limit rates (20%) in Zhangjiakou and Baoding.

2) Average contents were: Sichuan (0.24, 5.01, 3.67 mg/kg), Shandong (0.26, 4.19, 2.61 mg/kg), and Hebei (0.25, 5.66, 3.25 mg/kg). According to National Hygienic Standards, all Cd levels and all metals in Shandong were within limits, while Cr and Pb in Sichuan (9.38% and 17.19%) and Hebei (6.73% and 14.42%) exceeded limits. Hebei' s average Cr and Pb levels were significantly higher than Shandong' s. Shandong showed lightest contamination with no exceedances, while Sichuan and Hebei had Cr and Pb exceedances, with Pb being most serious.

3) Metal contents varied significantly by farm scale, with large-scale farms significantly lower than small-scale farms.

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Note: Figure translations are in progress. See original paper for figures.