

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

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Abstract

This study aimed to determine the concentrations of cadmium (Cd), chromium (Cr), and lead (Pb) in compound feed for laying hens during peak production in farms from Sichuan, Shandong, and Hebei provinces (including the Beijing-Tianjin-Hebei region), and to assess the contamination levels of these three heavy metals in major layer-producing provinces. A total of 268 compound feed samples were collected from 67 farms across Sichuan, Shandong, and Hebei provinces, and analyzed for Cd, Cr, and Pb content using high-resolution continuum source flame atomic absorption spectrometry (HR-CS FAAS). The results demonstrated: 1) In Sichuan province, the cities with maximum detected values for Cd, Cr, and Pb in layer compound feed were Chengdu, Leshan, and Leshan, respectively, with maximum values of 0.48, 15.94, and 14.12 mg/kg; the cities with 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 maximum detected values for Cd, Cr, and Pb were Qingdao and Rizhao, Heze, and Rizhao, respectively, with maximum values of 0.45, 5.88, and 4.11 mg/kg. 3) In Hebei province, the cities with maximum detected values for Cd, Cr, and Pb were Zhangjiakou, Zhangjiakou, and Baoding, respectively, with maximum values of 0.45, 15.38, and 8.38 mg/kg; the cities with highest exceedance rates for Cr and Pb were Zhangjiakou and Baoding, both with exceedance rates of 20%. 4) The average concentrations of Cd, Cr, and Pb in layer 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 Feed Hygiene Standard, Cd concentrations in all three provinces and Cd, Cr, and Pb concentrations in Shandong province were within permissible limits; the exceedance rates for Cr and Pb in Sichuan and Hebei

provinces were 9.38%, 17.19% and 6.73%, 14.42%, respectively. 5) The average Cr and Pb concentrations in layer compound feed from Hebei province were significantly higher than those from Shandong province ($P < 0.05$); the average Cd, Cr, and Pb concentrations in compound feed from large-scale farms were significantly lower than those from small-scale farms ($P < 0.05$). In conclusion, Cd, Cr, and Pb concentrations in layer compound feed from Shandong province complied with the standard. While Cd concentrations in Sichuan and Hebei provinces did not exceed the standard, they posed a potential exceedance risk; both Cr and Pb concentrations exceeded the standard to varying degrees, with Pb showing the most severe exceedance and warranting serious 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 concentrations of cadmium (Cd), chromium (Cr), and lead (Pb) in compound feed for laying hens during peak production in major poultry-producing provinces (Sichuan, Shandong, and Hebei) and assess the contamination levels of these three heavy metals. A total of 268 compound 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, 15.94, and 14.12 mg/kg, occurring in Chengdu (Cd) and Leshan (Cr and Pb). The highest over-limit rates for Cr and Pb were observed in Leshan (30%) and Chongzhou (50%), respectively. 2) In Shandong, the maximum detected values were 0.45 mg/kg Cd (Qingdao and Rizhao), 5.88 mg/kg Cr (Heze), and 4.11 mg/kg Pb (Rizhao). 3) In Hebei, the maximum values were 0.45 mg/kg Cd (Zhangjiakou), 15.38 mg/kg Cr (Zhangjiakou), and 8.38 mg/kg Pb (Baoding), with Zhangjiakou and Baoding showing the highest over-limit rates (20% each) for Cr and Pb. 4) Mean concentrations across the three provinces 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) 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% (Sichuan) and 6.73% and 14.42% (Hebei). Mean Cr and Pb levels in Hebei were significantly higher than in Shandong ($P < 0.05$), and large-scale farms showed significantly lower Cd, Cr, and Pb concentrations than small-scale farms ($P < 0.05$). In conclusion, Shandong's compound feed showed no heavy metal exceedances. While Cd levels in Sichuan and Hebei were within limits but approached threshold values, Cr and Pb showed varying degrees of exceedance, with Pb contamination being the most severe and warranting immediate attention.

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

Introduction

Eggs are highly nutritious livestock products with substantial production and consumption in China. However, egg safety incidents have occurred frequently, including the Sudan Red incident in 2005, the toxic egg event in the United States in 2013, and the counterfeit egg scandal in Germany in 2014. Cadmium (Cd), chromium (Cr), and lead (Pb) are three highly toxic heavy metals that have accumulated in air, soil, and water due to modern industrial and agricultural development, subsequently contaminating crops and feed ingredients. Additionally, some manufacturers use inferior mineral supplements to reduce costs, further contributing to heavy metal exceedances in compound feed. 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 are difficult to eliminate from feed and eggs. Therefore, assessing Cd, Cr, and Pb contamination in compound feed from major poultry-producing provinces (Sichuan, Shandong, and Hebei) is critical for evaluating egg safety and feed quality in these regions.

Detection technologies for Cd, Cr, and Pb include conventional sharp-line source atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS), and high-resolution continuum source atomic absorption spectrometry (HR-CS FAAS). Traditional AAS cannot simultaneously determine multiple elements and is now less commonly used, while ICP-MS remains expensive and inaccessible. HR-CS FAAS has gained popularity due to its multi-element capability and relatively lower cost. Meng et al. used wet digestion-flame AAS to analyze trace elements in feed and eggs from a Henan poultry farm, finding both feed and corresponding eggs exceeded National Hygienic Standards for Pb and Cd. Yuan et al. employed ICP-MS to determine 13 heavy metals in Sichuan's layer feed and eggs, revealing Cd levels within limits (0.5 mg/kg) but Cr and Pb (10 and 5 mg/kg) exceedances at rates of 11.1% and

18.2%, respectively. Tu et al. analyzed 96 aquafeed samples from Fujian, finding Pb within safe limits (5 $\mu\text{g/g}$) but Cd and Cr exceedances in some samples, with severe Cr contamination. Wang et al. investigated heavy metals in livestock feed in North China, noting significant variation across farms, with pig and beef cattle feed showing the most severe contamination, followed by broiler and layer feed. While Cd remained within limits across all samples, Cr and Pb showed varying exceedance rates, with layer feed Pb at 53.85%. Zhu et al. analyzed heavy metals in fattening pig feed from 64 large-scale farms in Shaanxi, finding Cd, Cr, and Pb all exceeded national standards by up to 110.86, 5.44, and 7.67 times, respectively.

Overall, feed heavy metal contamination is severe and shows an increasing accumulation trend in poultry eggs. Sichuan, Shandong, and Hebei are major livestock provinces with developed modern industries and serious environmental pollution, posing threats to healthy animal production. However, few studies have mapped the regional distribution of these three heavy metal risk factors across the three provinces. This study therefore employed HR-CS FAAS to simultaneously determine Cd, Cr, and Pb concentrations in compound feed from laying hen farms in Sichuan, Shandong, and Hebei, assessing contamination levels to provide a data foundation for feed and egg safety evaluation.

Materials and Methods

1.1 Sample Collection

Compound feed samples were collected from 67 laying hen farms of different scales in Sichuan, Shandong, and Hebei provinces (including Beijing-Tianjin-Hebei region, collectively referred to as Hebei) between January and August 2016. Four 500 g samples were collected from each farm, totaling 268 samples. Following the Feed Sampling standard (GB/T 14699.1-2005), samples were obtained from 16 farms in Sichuan (covering 1 provincial capital and 6 prefecture-level cities across eastern, western, southern, and northern regions, $n=64$), 25 farms in Shandong (1 provincial capital and 24 prefecture-level cities, $n=100$), and 26 farms in Hebei (4 prefecture-level cities and 2 surrounding cities: Tianjin's Jixian and Beijing's Daxing and Yanqing districts, $n=104$). 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 concentrations in compound feed were determined by HR-CS FAAS. Feed samples were ground through a 40-mesh sieve, and 0.5 g was accurately weighed into polytetrafluoroethylene digestion vessels. After adding 8 mL nitric acid (HNO_3) and 2 mL hydrogen peroxide, samples were pre-digested for 30 minutes in a constant temperature heater, then digested using a microwave digestion system. Digests were diluted to 25 mL with 0.5% (v/v) HNO_3 solution and mixed thoroughly for analysis. HR-CS FAAS operating conditions are detailed in Table 2.

1.3 Instruments and Reagents

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

Reagents: HNO₃ 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 as required; ultrapure water (18.2 MΩ · cm) from a Millipore system (USA).

1.4 Statistical Analysis

Data were analyzed using SPSS 19.0 software. One-way ANOVA was performed, and Duncan's multiple range test was used to compare differences among regions (Sichuan, Shandong, Hebei) and farm scales. Significance was set at $P < 0.05$. Results are expressed as mean \pm standard deviation.

Results

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

As shown in Table 3, simultaneous determination of Cd, Cr, and Pb in one feed sample required only 2 hours. Recovery rates ranged from 96.0-101.0% for Cd, 98.6-99.9% for Cr, and 95.4-101.3% for Pb. Detection limits (LOD) were 1.7, 19.7, and 34.6 ng/mL for Cd, Cr, and Pb, respectively. These results demonstrate that HR-CS FAAS is accurate, rapid, and suitable for determining Cd, Cr, and Pb in compound feed.

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

Tables 4, 5, and 6 present the heavy metal distribution in 64 feed samples from 7 cities in Sichuan. Cd levels did not exceed limits, with the maximum value (0.48 mg/kg) found in Chengdu. Cd concentrations in Chengdu, Deyang, Leshan, and Ya'an approached the national limit (0.5 mg/kg), indicating contamination risk. Cr and Pb levels remained within limits in Chengdu, Deyang, Mianyang, Nanchong, and Ya'an. However, both metals exceeded standards in Leshan, with over-limit rates of 30% (Cr) and 45% (Pb), mean exceedance values of 13.45 and 8.67 mg/kg, and maximum detected values of 15.94 and 14.12 mg/kg, respectively. Pb also exceeded limits in Chongzhou at a 50% over-limit rate (mean: 5.59 mg/kg). Overall, maximum values for Cd, Cr, and Pb occurred in Chengdu, Leshan, and Leshan, respectively, while the highest over-limit rates for Cr and Pb were in Leshan and Chongzhou.

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

Analysis of 100 feed samples from 9 cities in Shandong (Tables 7 , 8 , and 9) revealed that all Cd, Cr, and Pb concentrations were within national limits, indicating relatively light contamination. The maximum Cd value (0.45 mg/kg) occurred in Qingdao and Rizhao, with the highest mean concentrations in Yantai (0.31 mg/kg) and Rizhao (0.34 mg/kg). For Cr, maximum values were detected in Heze (6.17 mg/kg) and Dezhou (5.88 mg/kg), with highest means in Heze (5.42 mg/kg) and Liaocheng (4.95 mg/kg). Pb maximum values were found in Yantai (3.88 mg/kg) and Rizhao (4.11 mg/kg), 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

In 104 feed samples from 6 cities in Hebei (Tables 10 , 11 , and 12), Cd levels were within limits, with maximum values in Zhangjiakou (0.45 mg/kg) and Baoding (0.44 mg/kg). Mean Cd concentrations followed the order: Zhangjiakou > Baoding > Gaobeidian > Langfang > Tianjin > Beijing. Cr and Pb exceeded limits in Zhangjiakou and Baoding. Cr maximum values were 18.35 mg/kg (Zhangjiakou) and 15.38 mg/kg (Baoding), exceeding the 10 mg/kg limit with over-limit rates of 20.00% and 7.50%, respectively (mean exceedance values: 8.38 and 7.32 mg/kg). Pb maximum values were 8.38 mg/kg (Zhangjiakou) and 7.32 mg/kg (Baoding), 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, followed by Baoding.

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

Table 13 shows no significant differences in Cd concentrations among provinces ($P > 0.05$), with means ranking: Shandong > Hebei > Sichuan, all below the 0.5 mg/kg limit. Cr and Pb exceeded limits in Sichuan (9.38% and 17.19%) and Hebei (6.73% and 14.42%), with mean exceedance values of 13.45 and 8.11 mg/kg (Sichuan) and 15.55 and 6.93 mg/kg (Hebei). Sichuan's Cr levels did not differ significantly from Shandong or Hebei ($P > 0.05$), while Hebei's mean Cr concentration was significantly higher than Shandong's ($P < 0.05$). Mean Pb concentrations 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, operations were categorized as small (<10,000 birds), medium-small (10,000-20,000), medium (20,000-50,000), and large (>100,000) scale. Table 14 reveals significant variation in heavy metal concentrations across scales. Cr and Pb exceeded limits in small, medium-small, and medium-scale

farms, but not in large-scale farms. Cd and Cr concentrations generally decreased with increasing farm size: small > medium-small > medium > large, with small-scale farms significantly higher than large-scale ($P < 0.05$). Pb concentrations were highest in medium-scale and lowest in large-scale farms, with medium, small, and medium-small farms significantly exceeding large-scale farms ($P < 0.05$). Pb showed the highest over-limit rates and most severe contamination, particularly in small and medium-small farms.

Discussion

3.1 Contamination Levels and Distribution in Compound Feed

Heavy metal concentrations 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 techniques. One farm's Pb level exceeded the U.S. Mineral Tolerance of Animals limit (10 mg/kg), likely due to inferior raw materials and additives and non-standard self-formulation practices. In contrast, industrially developed Chengdu, Deyang, and Mianyang showed no exceedances, while Ya'an with less heavy industry had low contamination levels. Overall, Sichuan's over-limit rates (Cd: 0%, Cr: 9.38%, Pb: 17.19%) were consistent with Yuan et al.'s findings (Cd: 0%, Cr: 11.1%) but showed higher Pb exceedance (17.19% vs. 0%), possibly due to larger sample sizes and more small-scale farm sources compared to their study of commercial feed manufacturers.

Shandong showed 100% detection rates but no exceedances, indicating lighter contamination than Sichuan and Hebei. Despite predominantly small-medium scale sampling, all metals remained within limits, likely reflecting Shandong's feed industry characteristics: shrinking self-formulation markets, elimination of 20% of small/obsolete enterprises improving overall quality, expansion of large enterprises, and improved small-medium enterprise efficiency due to low raw material prices and recovered farming conditions. Additionally, Shandong's environmental reports indicate pollution primarily involves atmospheric and water contaminants (particulate matter and sulfur dioxide) rather than heavy metals.

In Hebei, Cr and Pb exceedances concentrated in Zhangjiakou and Baoding, possibly due to intensive livestock operations contaminating soil and water through manure discharge, creating a vicious cycle. These cities are also key chemical industry areas with environmental soil, water, and air pollution. Beijing samples showed no exceedances, likely because they came from large-scale farms in ecological conservation and new urban development zones. Wang et al. reported higher exceedance rates for North China (including Shandong and Hebei) at 0% (Cd), 34.86% (Cr), and 53.85% (Pb), possibly due to broader regional coverage, complex sample sources from polluted areas, and less regulatory oversight two years prior.

Comprehensively, Shandong's feed showed no exceedances of National Hygienic

Standards, while Sichuan and Hebei exhibited varying Cr and Pb exceedances significantly higher than Shandong. Cd approached limit values across all provinces, posing exceedance risks. Cr and Pb exceedances in Sichuan and Hebei likely relate to samples 主要来自中、小型养殖场 and high environmental heavy metal levels in soil and water.

Farm scale analysis revealed decreasing Cd, Cr, and Pb concentrations with increasing scale: small > medium-small > medium > large, with large-scale farms significantly lower than small-scale ($P < 0.05$). Small and medium-small farms lack scientific, scaled ingredient sourcing, causing severe contamination at the source. Excessive mineral additive use in self-formulated feed from small-medium farms also contributes, as does improper machinery and equipment use during processing, transport, and storage.

3.2 Potential Pollution Sources

Heavy metal contamination sources in layer feed are extensive, primarily including animal/plant ingredients and mineral additives. Animal-derived ingredients (bone meal, leather protein powder, fish meal) contain excessive Cd, Cr, and Pb, particularly adulterated fish meal. Plant ingredients (corn, corn by-products, wheat, DDGS, cottonseed meal) also show varying exceedances, with Cr and Pb being severe in some materials, especially corn and wheat bran. Excessive mineral additive supplementation causes contamination; feed-grade phosphates are primary Cr sources, with defluorinated phosphate and calcium dihydrogen phosphate averaging 110 and 83 mg/kg Cr, respectively, while feed-grade copper sulfate may contain 640 mg/kg Pb. Modern industrial development, particularly uncontrolled emissions from mining and smelting operations, and agricultural pesticide/fertilizer misuse also contaminate feed ingredients. Additionally, improper machinery and equipment use during feed processing contributes to heavy metal contamination.

3.3 Hazards of Cd, Cr, and Pb Contamination

Cd, Cr, and Pb exhibit multi-system and multi-organ toxicity with carcinogenic, teratogenic, and mutagenic effects. Cd accumulates slowly, causing chronic nephrotoxicity (impaired tubular reabsorption, proteinuria, aminoaciduria, glucosuria) and reducing layer performance, immunity, and egg quality. Cr exists as Cr^{3+} and Cr^{6+} ; Cr^{3+} aids insulin function and is essential for glucose and cholesterol metabolism, while Cr^{6+} is toxic, affecting redox processes, precipitating nucleic acids, and disrupting enzyme systems. Research shows low Cr doses (<10 mg/kg) improve egg production and quality (shell thickness and strength), while high doses (>600 mg/kg) reduce hatchability and damage liver and kidneys. Pb primarily damages hematopoietic organs and kidneys, with additional toxicity to respiration, immunity, and embryonic development. 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. Jeng et al. found that layers consuming

water with 1.3 mg/kg Cd and 6.7 mg/kg Pb showed significantly reduced body weight, egg production, and egg weight. Human consumption of contaminated eggs can impair infant and adolescent intellectual development, hearing, kidney function, and cardiovascular health.

This study found Cd concentrations approaching limit values across provinces, posing serious risks through long-term accumulation. Cr and Pb exceedances in Sichuan (9.38% and 17.19%) and Hebei (6.73% and 14.42%) were significant, with small-scale farms showing significantly higher contamination than large-scale operations. Therefore, feed manufacturers and regulatory agencies in all three provinces must prioritize regular monitoring of Cd, Cr, and Pb in compound feed and eggs. Given complex ingredient sources and potential batch-to-batch variation, further analysis of multiple batches is needed to clarify dynamic contamination patterns and distribution.

Conclusions

- In Sichuan, maximum Cd, Cr, and Pb values occurred in Chengdu, Leshan, and Leshan (0.48, 15.94, and 14.12 mg/kg), with highest Cr and Pb over-limit rates in Leshan (30%) and Chongzhou (50%). In Shandong, maximum values were in Qingdao/Rizhao (Cd: 0.45 mg/kg), Heze (Cr: 5.88 mg/kg), and Rizhao (Pb: 4.11 mg/kg). In Hebei, maximum values were in Zhangjiakou (Cd: 0.45 mg/kg; Cr: 15.38 mg/kg) and Baoding (Pb: 8.38 mg/kg), with Zhangjiakou and Baoding showing highest Cr and Pb over-limit rates (20%).
- Mean concentrations 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). All Cd levels and Shandong's metals were within National Hygienic Standards. Sichuan and Hebei showed Cr and Pb exceedances at rates of 9.38%/17.19% and 6.73%/14.42%, respectively. Hebei's mean Cr and Pb levels were significantly higher than Shandong's. Shandong showed lightest contamination with no exceedances, while Sichuan and Hebei had severe Cr and Pb pollution, particularly Pb, requiring urgent attention.
- Heavy metal concentrations varied significantly by farm scale, with large-scale farms significantly lower than small-scale farms for Cd, Cr, and Pb.

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