# Distribution of Heavy Metals in Commonly Used Energy and Protein Feeds in Sichuan Province (Postprint)

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## Abstract

This study aimed to investigate the distribution of heavy metals in commonly used energy and protein feeds in Sichuan Province, providing a scientific basis for the safe production of feed and livestock products. A total of 68 energy feed samples [including corn, wheat, dried distillers grains with solubles (DDGS), sprayed corn bran, wheat bran, and rice bran] and 61 protein feed samples (including corn gluten meal, corn germ meal, soybean meal, rapeseed meal, and cottonseed meal) were collected from Sichuan Province. The contents of manganese (Mn), copper (Cu), zinc (Zn), vanadium (V), chromium (Cr), cobalt (Co), nickel (Ni), selenium (Se), molybdenum (Mo), cadmium (Cd), arsenic (As), mercury (Hg), and lead (Pb) were determined using inductively coupled plasma mass spectrometry (ICP-MS). The results showed: 1) In corn, the exceedance rates for Cu, Cr, and Pb were 5.00%, 20.00%, and 5.00%, respectively, with average values of non-compliant samples being 11.00, 1.41, and 1.06 mg/kg, respectively; in wheat, the exceedance rates for Cu, Zn, Cr, Se, and Pb were 50.00%, 30.00%, 50.00%, 20.00%, and 30.00%, respectively, with average values of non-compliant samples being 12.96, 54.67, 4.92, 0.53, and 0.72 mg/kg, respectively. 2) In wheat bran, the exceedance rates for Cu, Zn, Cr, Se, Cd, and Pb were 100.00%, 54.55%, 81.82%, 9.09%, 45.45%, and 72.73%, respectively, with average values of non-compliant samples being 16.01, 62.00, 13.96, 0.82, 0.42, and 1.99 mg/kg, respectively; in rice bran, the exceedance rates for Cu, Zn, Cr, As, Se, Cd, and Pb were 83.33%, 16.67%, 83.33%, 83.33%, 16.67%, 66.67%, and 100.00%, respectively, with average values of non-compliant samples being 11.48, 64.00, 5.37, 1.05, 0.32, 0.23, and 19.57 mg/kg, respectively. 3) In corn germ meal, the exceedance rates for Cu, Zn, Cr, As, Se, and Pb were 25.00%, 37.50%, 75.00%, 12.50%, 25.00%, and 50.00%, respectively, with average values of non-compliant samples being 13.50, 64.00, 6.10, 1.82, 0.46, and 1.66 mg/kg, respectively; in corn gluten meal, the exceedance rates for Cu, Cr, and Se were

11.11%, 55.56%, and 55.56%, respectively, with average values of non-compliant samples being 11.40, 1.56, and 0.94 mg/kg, respectively; in sprayed corn bran, the exceedance rates for Zn, Cr, and Pb were 44.44%, 77.78%, and 55.56%, respectively, with average values of non-compliant samples being 57.50, 11.75, 6.13, 5.57, and 1.65 mg/kg, respectively; in DDGS, the exceedance rates for Cu, Zn, Cr, Se, Cd, and Pb were 33.33%, 58.33%, 66.67%, 8.33%, 8.33%, and 66.67%, respectively, with average values of non-compliant samples being 11.75, 62.57, 5.57, 0.30, 0.62, and 4.47 mg/kg, respectively. 4) In soybean meal, the exceedance rates for Cr and Se were 15.00% and 25.00%, respectively, with average values of non-compliant samples being 3.90 and 1.63 mg/kg, respectively; in cottonseed meal, the exceedance rates for Cr, Se, and Pb were 40.00%, 30.00%, and 15.00%, respectively, with average values of non-compliant samples being 3.43, 1.18, and 3.14 mg/kg, respectively; in rapeseed meal, the exceedance rates for Cr, As, Se, Cd, and Pb were 81.82%, 18.18%, 45.45%, 9.09%, 45.45%, and 72.73%, respectively, with average values of non-compliant samples being 7.48, 2.22, 0.36, 0.24, and 4.91 mg/kg, respectively. Overall, grain processing by-products and rapeseed meal exhibited relatively serious heavy metal contamination, with Cr and Pb contents severely exceeding standards in some feed ingredients, posing potential risks that warrant attention.

## Full Text

## Preamble

### Investigation on Heavy Metal Distribution in Energy Feedstuffs and Protein Feedstuffs of Sichuan Province

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**Abstract:** This study was conducted to investigate the distribution of heavy metals in commonly used energy and protein feedstuffs in Sichuan Province, providing a scientific basis for safe feed and livestock product production. Sixty-eight samples of energy feedstuffs [including corn, wheat, distillers dried grains with solubles (DDGS), sprayed corn husk, wheat bran, and rice bran] and sixty-one samples of protein feedstuffs (including corn gluten meal, corn germ meal, soybean meal, rapeseed meal, and cottonseed meal) were collected from Sichuan Province. The contents of manganese (Mn), copper (Cu), zinc (Zn), vanadium (V), chromium (Cr), cobalt (Co), nickel (Ni), selenium (Se), molybdenum (Mo), cadmium (Cd), arsenic (As), mercury (Hg), and lead (Pb) were determined

using inductively coupled plasma mass spectrometry (ICP-MS). The results showed that: 1) The over-standard rates of Cu, Cr, and Pb in corn were 5.00%, 20.00%, and 5.00%, respectively, with mean values of over-standard samples at 11.00, 1.41, and 1.06 mg/kg. The over-standard rates of Cu, Zn, Cr, Se, and Pb in wheat were 50.00%, 30.00%, 50.00%, 20.00%, and 30.00%, respectively, with mean values of over-standard samples at 12.96, 54.67, 4.92, 0.53, and 0.72 mg/kg. 2) The over-standard rates of Cu, Zn, Cr, Se, Cd, and Pb in wheat bran were 100.00%, 54.55%, 81.82%, 9.09%, 45.45%, and 72.73%, respectively, with mean values of over-standard samples at 16.01, 62.00, 13.96, 0.82, 0.42, and 1.99 mg/kg. The over-standard rates of Cu, Zn, Cr, As, Se, Cd, and Pb in rice bran were 83.33%, 16.67%, 83.33%, 83.33%, 16.67%, 66.67%, and 100.00%, respectively, with mean values of over-standard samples at 11.48, 64.00, 5.37, 1.05, 0.32, 0.23, and 19.57 mg/kg. 3) The over-standard rates of Cu, Zn, Cr, As, Se, and Pb in corn germ meal were 25.00%, 37.50%, 75.00%, 12.50%, 25.00%, and 50.00%, respectively, with mean values of over-standard samples at 13.50, 64.00, 6.10, 1.82, 0.46, and 1.66 mg/kg. The over-standard rates of Cu, Cr, and Se in corn gluten meal were 11.11%, 55.56%, and 55.56%, respectively, with mean values of over-standard samples at 11.40, 1.56, and 0.94 mg/kg. The over-standard rates of Zn, Cr, and Pb in sprayed corn husk were 44.44%, 77.78%, and 55.56%, respectively, with mean values of over-standard samples at 57.50, 11.75, 6.13, 5.57, and 1.65 mg/kg. The over-standard rates of Cu, Zn, Cr, Se, Cd, and Pb in DDGS were 33.33%, 58.33%, 66.67%, 8.33%, 8.33%, and 66.67%, respectively, with mean values of over-standard samples at 11.75, 62.57, 5.57, 0.30, 0.62, and 4.47 mg/kg. 4) The over-standard rates of Cr and Se in soybean meal were 15.00% and 25.00%, respectively, with mean values of over-standard samples at 3.90 and 1.63 mg/kg. The over-standard rates of Cr, Se, and Pb in cottonseed meal were 40.00%, 30.00%, and 15.00%, respectively, with mean values of over-standard samples at 3.43, 1.18, and 3.14 mg/kg. The over-standard rates of Cr, As, Se, Cd, and Pb in rapeseed meal were 81.82%, 18.18%, 45.45%, 9.09%, and 72.73%, respectively, with mean values of over-standard samples at 7.48, 2.22, 0.36, 0.24, and 4.91 mg/kg. Overall, heavy metal pollution was more severe in grain processing by-products and rapeseed meal, with Cr and Pb contents in some feed ingredients significantly exceeding standards, posing potential risks that warrant attention.

**Keywords:** ICP-MS; feed ingredients; heavy metals; pollution

Heavy metals generally refer to metallic elements with a density greater than 4.5 g/cm$^3$, with 45 known heavy metal elements. The main types of environmental heavy metal pollution include copper (Cu), zinc (Zn), chromium (Cr), cobalt (Co), arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb). Heavy metal pollution in feed primarily refers to elements with significant biological toxicity such as Cd, Pb, As, and Hg.

With the excessive addition of some essential heavy metal elements (Cu, Zn, etc.) in feed, these elements have also become potential heavy metal pollutants in feed. Apart from deliberate over-addition (Cu, Zn, etc.), heavy metals in

feed mainly originate from contamination of feed ingredients and improper use of machinery and utensils during feed processing [1-2]. Due to the numerous toxic effects of heavy metals on animals and humans, once feed and feed ingredients are contaminated, the pollution is difficult to eliminate. Therefore, heavy metal contamination in feed ingredients, feed, and animal products should receive sufficient attention. China has established limit standards for Cr, Cd, Pb, Hg, and As contents in compound feed [5]. Tu et al. [3] found that Pb content in aquatic feed ingredient samples was $ $7.51 mg/kg, Hg content $ $0.476 mg/kg, As content $ $1.43 mg/kg, and Cd content $ $1.39 mg/kg. Xie et al. [4] reported that the over-standard rates of Cu and Zn in pig compound feed both reached 100%. However, except for rice bran, fish meal, phosphates, and limestone powder, there are no relevant standards for feed ingredients. The industry standard NY 861-2004 [6] sets limits for heavy metals in grains and their products, including Cu, Zn, Pb, Cd, As, Cr, selenium (Se), and Hg, covering various grains such as rice, flour, and corn. However, in recent years, with the widespread application of various grain processing by-products such as distillers dried grains with solubles (DDGS), corn germ meal, and corn husk, heavy metal pollution faces new risks. The primary approach to reducing heavy metal hazards to animals is prevention, i.e., reducing heavy metal content in feed and feed ingredients. Therefore, this study collected major feed ingredients used by major feed sales enterprises and farms in Sichuan Province to analyze their heavy metal content, aiming to further understand the pollution status and patterns of heavy metals in feed ingredients used in Sichuan Province, and to provide a scientific basis for strengthening supervision of feed ingredients, guiding enterprises in rational use of raw materials, and ensuring feed and livestock product safety.

### 1.1.1 Sampling Time and Location

Samples were collected from feed mills, farms (households), and grain distribution centers of different scales in Sichuan Province from July to September 2014, with the origin of feed recorded.

### 1.1.2 Sampling Requirements

Sampling was conducted strictly in accordance with the GB/T 14699.1-2005 “Feed Sampling” standard [7].

### 1.1.3 Sample Types and Quantities

The samples included energy feedstuffs: corn (20 samples), DDGS (12 samples), sprayed corn husk (9 samples), wheat (10 samples), wheat bran (11 samples), and rice bran (6 samples); and protein feedstuffs: soybean meal (20 samples), corn germ meal (8 samples), rapeseed meal (11 samples), corn gluten meal (9 samples), and cottonseed meal (13 samples), totaling 129 samples.

### 1.2 Instruments and Reagents

Ultrapure water preparation system (Millipore, USA), inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7500a, USA), microwave digestion furnace (Milestone ethos t, Italy), heavy metal standards (Sigma, USA), nitric acid (Merck, superior grade, Germany), and ultrapure water.

### 1.3 Detection Indicators

Heavy metals: manganese (Mn), Cu, Zn, vanadium (V), Cr, Co, nickel (Ni), Se, Mo, Cd, Hg, and Pb, and the metalloid As, totaling 13 elements.

### 1.4 Determination Method

Samples were digested using a microwave digestion furnace, and heavy metal contents were quantitatively detected by ICP-MS with reference to the methods of Wang et al. [8] and Huang et al. [9].

### 1.5 Limit Standards

The heavy metal limit standards referenced in this study are shown in Table 1 [3]. The overall sample mean, over-standard rate, mean of over-standard samples, and maximum values were calculated.

Table 1 Limited standard of heavy metal in foodstuffs and its products (NY861 –2004) mg/kg
Items Grains and its products Beans and its products
0.20 (rice) 0.10 (flour) 0.05 (corn)

### 1.6 Data Analysis

Excel 2007 was used for statistical data analysis, with results expressed as means and errors indicated as SEM, while coefficient of variation (CV, %) was also calculated. SPSS 19.0 was used for analysis of variance (ANOVA) on heavy metal contents in feed ingredients from different origins, with $P < 0.05$ considered statistically significant.

### 2.1 Overall Distribution of Heavy Metals in Feed Ingredients

As shown in Table 2 , the average contents of the 13 elements Mn, Cu, Zn, V, Cr, Co, Ni, As, Se, Mo, Cd, Hg, and Pb in these commonly used bulk feed ingredients (including corn, DDGS, sprayed corn husk, wheat, wheat bran, rice bran, corn gluten meal, corn germ meal, soybean meal, rapeseed meal, and cottonseed meal) were 41.79, 8.68, 40.28, 0.60, 3.36, 0.29, 1.29, 0.20, 0.45, 1.23, 0.06, 0.0049, and 1.87 mg/kg, respectively. The samples with the highest detected values of Mn and Pb were both rice bran from Southwest China, at 200.00 and 80.00 mg/kg, respectively. The samples with the highest values of Cu, Ni, and Mo were cottonseed meal from Northwest China, at 19.60, 5.20, and 8.00

mg/kg, respectively. The samples with the highest values of Zn and As were rapeseed meal from Southwest China, at 86.00 and 3.80 mg/kg, respectively. The sample with the highest V value was corn germ meal (5.80 mg/kg) from Northeast China. The samples with the highest values of Cr and Cd were both wheat bran, from North China (56 mg/kg) and Southwest China (1.12 mg/kg), respectively. The sample with the highest Co value was DDGS (8.60 mg/kg) from Southwest China. The sample with the highest Se value was soybean meal (14.80 mg/kg) from North China.

### 2.2 Distribution of Heavy Metals in Feed Ingredients from Different Regions

The raw material samples were collected from six major regions of China: Southwest, Northwest, North, Central, East, and Northeast. As shown in Table 3 , the contents of heavy metals in feed ingredients from different regions showed varying degrees of difference. The contents of Cu, Zn, Co, Mo, Cr, and Cd did not differ significantly among regions ($P > 0.05$). The Mn content in feed ingredients from Southwest, North, and Central China was significantly higher than that from Northwest and East China ($P < 0.05$). The Se content in feed ingredients from Southwest China was significantly higher than that from Northwest, Central, East, and Northeast China ($P < 0.05$). The Ni content in feed ingredients from North China was significantly higher than that from all other regions ($P < 0.05$). The As content in feed ingredients from Southwest, Northwest, and Central China was significantly higher than that from North China ($P < 0.05$). The Pb and Hg contents in feed ingredients from Southwest China were both significantly higher than those from all other regions ($P < 0.05$). These data indicate that heavy metal pollution in feed ingredients from Southwest China is relatively severe.

Table 3 Heavy metal distribution in feed ingredients from different regions mg/kg

| Items | Southwest | Northwest | North | Central | East | Northeast | P-value |
|---|---|---|---|---|---|---|---|
| Mn | 54.56a | 23.94b | 51.84a | 82.77a | 28.72b | 43.26ab | |
| Cu | 1.16a | 0.28b | 1.42a | 1.04a | 0.93a | 0.01a | |
| Zn | 3.68a | 1.04a | 0.00b | 0.44b | 0.80ab | 2.56b | |
| Se | 1.96b | 0.00b | 0.51b | 0.09b | 0.40a | 0.57a | |
| Co | 0.00b | 0.30b | 0.40b | 1.01a | 0.55b | 1.15a | |
| Ni | 1.60ab | 1.36ab | 0.00b | 0.16b | 0.01b | 1.06b | |
| Mo | 0.40a | 0.57a | 0.00b | 0.30b | 0.40b | 1.01a | |
| Cr | 0.55b | 1.15a | 1.60ab | 1.36ab | 0.00b | 0.16b | |
| As | 0.01b | 0.51b | 0.09b | 0.40a | 0.57a | 0.00b | |
| Cd | 0.30b | 0.40b | 1.01a | 0.55b | 1.15a | 1.60ab | |
| Hg | 1.36ab | 0.00b | 0.16b | 0.01b | 1.06b | | |
| Pb | 0.40b | 1.01a | 0.55b | 1.15a | 1.60ab | | |

In the same row, values with different lowercase letter superscripts indicate significant differences ($P < 0.05$), while values with the same or no letter super-

scripts indicate no significant difference ($P > 0.05$).

### 2.3 Distribution of Heavy Metals in Grain Feedstuffs and Their By-products

The distribution of various heavy metals in grain feedstuffs and by-product feedstuffs is shown in Tables 4 , 5 , and 6 . According to the grain and its products regulations in Table 1 , the over-standard rates of Cu, Cr, and Pb in corn were 5.00%, 20.00%, and 5.00%, respectively, with mean values of over-standard samples at 11.00, 1.41, and 1.06 mg/kg, slightly above the standards. The coefficient of variation for Pb (2,101.16%) was extremely large, with one sample containing Pb as high as 1.06 mg/kg. In wheat, the over-standard rates of Cu, Zn, Cr, Se, and Pb were 50.00%, 30.00%, 50.00%, 20.00%, and 30.00%, respectively. The mean value of over-standard samples for Cr was 4.92 mg/kg, exceeding the standard by 3.92-fold, and the overall sample mean was 2.77 mg/kg, exceeding the standard by 1.77-fold, indicating a serious situation. The mean values of other over-standard indicators in wheat were slightly above the standards, while the overall sample means did not exceed the standards.

In wheat bran, the over-standard rates of Cu, Zn, Cr, Se, Cd, and Pb were 100.00%, 54.55%, 81.82%, 9.09%, 45.45%, and 72.73%, respectively. The mean value of over-standard samples for Cr was 13.96 mg/kg, 13.96 times the standard (with two samples as high as 56.00 and 52.00 mg/kg, both from Henan Province). The mean value of over-standard samples for Pb was 1.99 mg/kg, nearly 4 times the standard. The overall sample means for both Cr and Pb (11.45 and 1.60 mg/kg) also exceeded the standards. The mean values of over-standard samples for Cu, Zn, Cd, and Se were slightly above the standards, while the overall sample means did not exceed the standards. The heavy metal contamination situation in rice bran was similar to that in wheat bran, with Cu, Zn, Cr, As, Se, Cd, and Pb all exceeding standards to varying degrees. The over-standard rates were 83.33%, 16.67%, 83.33%, 83.33%, 16.67%, 66.67%, and 100.00%, respectively. The mean value of over-standard samples for Pb was as high as 19.57 mg/kg, nearly 50 times the standard, with one sample (from Hebei Province) reaching 80.00 mg/kg, 200 times the standard. The mean value of over-standard samples for Cr was 5.37 mg/kg, 5.37 times the standard. The overall sample means for these two elements (4.55 and 19.57 mg/kg) also far exceeded the standards. The mean values of over-standard samples for other elements were slightly above the standards, while the overall sample means did not exceed the standards. Except for Se, Mn, and Co, which were relatively evenly distributed, the coefficients of variation for other samples were relatively high.

In corn germ meal, a corn processing by-product, Cu, Zn, Cr, As, Se, and Pb all exceeded standards to varying degrees, with over-standard rates of 25.00%, 37.50%, 75.00%, 12.50%, 25.00%, and 50.00%, respectively. Except for Cr and Pb, whose mean values of over-standard samples (6.10 and 1.66 mg/kg) were far above the standards, the mean values of other over-standard elements were

slightly above the standards. Meanwhile, the overall sample means for Cr and Pb (4.61 and 0.87 mg/kg) also exceeded the standards. The distribution of all heavy metals in corn germ meal was uneven, with large coefficients of variation.

In corn gluten meal, the over-standard rates of Cu, Cr, and Se were 11.11%, 55.56%, and 55.56%, respectively. The mean value of over-standard samples for Se was 0.94 mg/kg, 3.13 times the standard. The overall sample means for Cr and Se (1.03 and 0.62 mg/kg) also exceeded the standards. The coefficients of variation for Cd and Hg were relatively small at 9.37% and 14.57%, respectively, while the distribution of other indicators was uneven with large coefficients of variation.

In sprayed corn husk, the over-standard rates of Zn, Cr, and Pb were 44.44%, 77.78%, and 55.56%, respectively, with mean values of over-standard samples at 57.50, 11.75, 6.13, 5.57, and 1.65 mg/kg. In DDGS, Cu, Zn, Cr, Se, Cd, and Pb all exceeded standards to varying degrees, with over-standard rates of 33.33%, 58.33%, 66.67%, 8.33%, 8.33%, and 66.67%, respectively. Except for Cr, Cd, and Pb, whose mean values of over-standard samples (5.57, 0.62, and 4.47 mg/kg) were relatively high, the mean values of other over-standard indicators were slightly above the standards. Meanwhile, the overall sample means for these three elements (3.84, 0.07, and 3.01 mg/kg) also exceeded the standards.

These results demonstrate that pollution in processing by-products such as corn germ meal, DDGS, wheat bran, and rice bran was more severe than in their original grains (corn, wheat), with higher numbers of over-standard elements, degrees of excess, and average contents.

Table 4 Heavy metal distribution in grain feed ingredients
Items Sample number Over-standard rate/% Mean of over-standard sample/(mg/kg) Mean of total sample/(mg/kg) Maximum/(mg/kg)
Wheat Over-standard rate/% Mean of over-standard sample/(mg/kg) Mean of total sample/(mg/kg) Maximum/(mg/kg)
“—” indicates no relevant standard. The same as below.

Table 5 Heavy metal distribution in wheat bran and rice bran
Items Sample number Wheat ingredients Over-standard rate/% Mean of over-standard sample/(mg/kg) Mean of total sample/(mg/kg) Maximum/(mg/kg)
Rice bran ingredients

Table 6 Heavy metal distribution in corn processing by-products
Items Sample number Corn bran with syrup Corn germ meal Over-standard rate/% Mean of over-standard sample/(mg/kg) Mean of total sample/(mg/kg) Maximum/(mg/kg) Over-standard rate/% Mean of over-standard sample/(mg/kg) Mean of total sample/(mg/kg) Maximum/(mg/kg)

### 2.4 Distribution of Heavy Metals in Oilseed Meal Feedstuffs

The distribution of heavy metals in oilseed meal feedstuffs is shown in Table 7 . According to the beans and their products regulations in Table 1 , only Cr

and Se exceeded standards in soybean meal, with over-standard rates of 15.00% and 25.00%, respectively. The mean value of over-standard samples for Se was 3.90 mg/kg, 12 times the limit standard, and the overall sample mean was 1.14 mg/kg, still nearly 3 times the standard (with one sample containing 14.80 mg/kg Se from Chongqing). Except for Zn, Cr, and Hg, which had relatively small coefficients of variation and more even distribution, other elements were unevenly distributed with large coefficients of variation.

In cottonseed meal, the over-standard rates of Cr, Se, and Pb were 40.00%, 30.00%, and 15.00%, respectively, with mean values of over-standard samples at 3.43, 1.18, and 3.14 mg/kg, all nearly 3 times the standard, and overall sample means also exceeded the standards. All elements showed large coefficients of variation, particularly Pb (286.19%), with one sample as high as 7.44 mg/kg from Xinjiang.

In rapeseed meal, the over-standard rates of Cr, As, Se, Cd, and Pb were 81.82%, 18.18%, 45.45%, 9.09%, and 72.73%, respectively. Except for Se and Cd, which had lower over-standard rates with mean values of over-standard samples only 1.2 times the standard, other indicators had higher over-standard rates, with mean values of over-standard samples ranging from 4.44 to 7.48 times the standard. The mean value of over-standard samples for Cr was 7.48 mg/kg, 7.48 times the standard (with two samples as high as 15.40 and 10.40 mg/kg, both from Sichuan). The maximum Pb value was 22.00 mg/kg, 27.50 times the standard, and this sample was also from Sichuan Province. The overall sample means for Cr and Pb (6.25 and 3.58 mg/kg) also exceeded the standards, with coefficients of variation of 111.32% and 179.52%.

Table 7 Heavy metal distribution in oilseed meal feedstuffs
Items Sample number Soybean Cottonseed Over-standard rate/% Mean of over-standard sample/(mg/kg) Mean of total sample/(mg/kg) Maximum/(mg/kg) Over-standard rate/% Mean of over-standard sample/(mg/kg) Mean of total sample/(mg/kg) Maximum/(mg/kg)
Rapeseed Over-standard rate/% Mean of over-standard sample/(mg/kg) Mean of total sample/(mg/kg) Maximum/(mg/kg)

### 3.1 Heavy Metal Pollution in Different Feed Ingredients

The sources of heavy metal pollution mainly include atmosphere, water, and soil, with grain contamination by heavy metals primarily caused by soil. In recent years, the area of wastewater irrigation in China has continuously expanded, causing long-term and irreversible pollution to soil [10]. Meanwhile, the diversification of feed ingredients also poses new challenges to feed heavy metal safety.

Corn and wheat are the most commonly used feed ingredients. In this study, the Cu and Zn contents in corn were 2.57 and 25.22 mg/kg, respectively, slightly lower than results from other researchers. For example, Jin et al. [11] reported that the average Cu content in corn from a village in the Dachang mining area

of Guangxi was 2.90 mg/kg, and the average Zn content was 36.06 mg/kg. This is because the latter corn samples were from mining areas, where long-term natural stacking of waste rock and slag from mining, mineral processing, and smelting has caused serious heavy metal pollution to the environment [7]. In contrast, the corn in this study came from wide-ranging origins, possibly from both non-mining and mining areas. The As contents in corn and wheat in this study were similar to those reported by Xiao et al. [12] for grain and oil crops (grains and legumes) in China (mean value of 0.294 mg/kg). However, it is worth noting that the latter samples were from polluted areas where crops were severely contaminated by heavy metals, thus reflecting from another perspective that As pollution is intensifying.

There are few reports on heavy metal content in grain processing by-products. This study found that the content and over-standard rate of most heavy metal elements in by-products were higher than in their original crops. For example, pollution in wheat bran was more severe than in wheat, and pollution in sprayed corn husk was more severe than in corn. This indicates that heavy metal contamination may occur during the processing of raw materials, particularly Cr, Cd, and Pb from machine surfaces.

Essential trace elements such as Mn, Cu, Zn, and Se were relatively high in oilseed meals, which may be related to crop species. Notably, the Se content in soybean meal (1.14 mg/kg) was nearly 3 times higher than the limit standard (0.30 mg/kg).

Rapeseed meal showed severe contamination by harmful heavy metals, with over-standard rates for Cr and Pb exceeding 70%, and other harmful heavy metal elements also at relatively high levels. This may be related to the pressing process of rapeseed meal.

### 3.2 Potential Hazards of Heavy Metal Pollution in Feed

Mn, Cu, Zn, and Se are essential trace elements for animal growth and are commonly added in feed formulations. The Mn content in most feed ingredients in this study was relatively high, with wheat bran and rice bran containing over 130.00 mg/kg Mn. Based on feed formulation proportions of corn (50%-60%), soybean meal (15%-30%), wheat bran (approximately 5%), etc., the Mn content in the formulation (excluding premix) would be 16-21 mg/kg, which is lower than the feeding standards for chickens and dairy cows but comparable to the standard for pigs [13-17], thus requiring additional supplementation. When Cu and Zn elements from this study were converted to formulation levels using the above calculation method, they were slightly lower than or roughly equivalent to feeding standards [13-17], requiring small amounts of additional supplementation. Moreover, investigations of compound feed have found high over-standard rates for Cu and Zn, mainly caused by feed mills adding high levels of copper and zinc.

Animals have low requirements for Se but are also more prone to toxicity. For

example, NRC (1998) specifies that the Se requirement for pigs is 0.15-0.30 mg/kg, and NRC (2001) specifies that the Se requirement for lactating dairy cows is 0.30 mg/kg. However, the toxic dose of Se for chickens, pigs, sheep, and beef cattle is only 2.00 mg/kg [13-17]. In this study, corn gluten meal and cottonseed meal had relatively high Se contents of 0.62 and 0.67 mg/kg, respectively, while other raw materials did not exceed 0.30 mg/kg. If a corn-soybean meal diet is formulated, the Se content would be 0.14-0.22 mg/kg, which theoretically meets animal requirements. However, in actual production, additional Se is often added to improve animal performance. Since Se is prone to causing toxicity, the Se content in feed ingredients must be considered during additional supplementation in practical production to prevent over-addition.

As, Cd, Cr, Hg, and Pb are the most hazardous heavy metals to the environment, animals, and humans. For example, excessive Cr can cause liver and kidney diseases in animals [1-2], while excessive Pb can cause acute and chronic conditions such as anemia, immune dysfunction, altered resistance, and nervous system damage [1-2]. This study found that feed ingredients were seriously contaminated by Cr and Pb, consistent with the results of Wang [18]. Wheat bran contained Cr as high as 11.45 mg/kg, and rapeseed meal contained Cr as high as 6.25 mg/kg. When converted to formulation levels, this would be 0.90-1.01 mg/kg, approaching or exceeding the limit standards, which should raise vigilance. Additionally, although Pb content was low in corn in this study, it was high in its by-products and even higher in rice bran and rapeseed meal, all exceeding the limit standards. Therefore, monitoring of these raw materials in formulations must be emphasized; otherwise, animal poisoning is highly likely.

Heavy metal elements such as V, Co, Ni, and Mo are also essential for animals, but animals have low requirements for them. This study found that the contents of these heavy metals in feed ingredients were relatively low, consistent with the results of Zhang [19] and Wang et al. [20] on wheat grains and Chinese medicinal materials. Although their contents are low, they are sufficient to meet animal growth needs without additional supplementation. Therefore, research and application of these elements are relatively limited compared to other heavy metals, but they should receive attention from a safety perspective.

### 3.3 Distribution of Heavy Metals in Feed Ingredients from Different Regions

The results of this study found that the coefficients of variation for heavy metal contents in most raw materials were relatively large, which may be closely related to different origins of raw materials. This study found that feed ingredients from Southwest and Central China were relatively severely contaminated by heavy metals. For example, Cd (0.10 mg/kg) and Pb (3.68 mg/kg) in Southwest China, and Cd (0.15 mg/kg) in Central China were all significantly higher than in other regions, consistent with the results of Shi et al. [21] on vegetables in southern China. Lu et al. [22] investigated and analyzed the current status of heavy metal (Hg, Pb, As, Cd, Cr) pollution in vegetable field soils in Guizhou

Province, finding severe soil heavy metal pollution in the region. Tong et al. [23] investigated the distribution of heavy metals in soil of crop bases in Chengdu, finding that Hg and Cd pollution reached the national tertiary standard, with lighter Cu pollution. In Chongqing, soil heavy metal Hg showed some pollution, Pb content decreased significantly, while Cd and As contents increased significantly [24]. Comprehensive analysis shows that these heavy metal elements are widely distributed in Southwest China with relatively high contents, possibly related to the heavy metal background values of soils in the region.

This study found that samples from Northeast China had relatively low heavy metal contents, which contradicts the results of Yu et al. [25] on soils in Northeast China. This may be related to the slowed industrial development in Northeast China in recent years.

Additionally, when conducting data analysis, this study also performed statistical analysis on raw material sales enterprises. The results showed that small enterprises were more severely contaminated by Hg, Cd, Pb, As, and other elements, especially Pb, possibly due to lax supervision of feed ingredients in small enterprises. Pollution in medium and large enterprises was less severe, but it is worth noting that As content in samples from large enterprises was also relatively high, and quality control management for As should be strengthened.

Heavy metal pollution is more severe in grain processing by-products and rapeseed meal, with Cr and Pb contents in some feed ingredients significantly exceeding standards, posing potential risks that warrant attention.

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