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ORIGINAL PAPER

Assessment of aflatoxin and toxic heavy metal contamination in poultry feed and feed ıngredients from Van, Türkiye*

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Abstract

The health of livestock for human consumption is closely associated with animal feed. About 70%-80% of the product cost of poultry farming in Türkiye is constituted by the cost of feed. For this reason, feed must not contain toxins, contaminants, heavy metals, and harmful microorganisms in order to raise animals on healthy feed and improve the management of poultry. The aim of this study was to assess aflatoxin and toxic heavy metal contamination in poultry feeds and feed ingredients in Van province. A total of 30 samples comprising poultry feeds (n=15) and poultry feed ingredients (n=15) were analyzed for the detection of aflatoxin B_1, B_2, G_1, G_2 and toxic heavy metals (Al, Ni, As, Cd, Pb). The concentrations of aflatoxin B_1, B_2, G_1, G_2 in the samples were determined using HPLC, and heavy metal analyses were determined using ICP-MS. Aflatoxin was detected in 9 of 30 samples, and the aflatoxin levels detected in 4 samples were above the maximum permissible limit as recommended by the Turkish Food Codex, EU regulations and FAO/WHO Codex Alimentarius. Different levels of aluminium, nickel, arsenic, cadmium and lead were detected in all samples in the study. They ranged from 1.097-19.046, 0.026-0.478, 0.0005-0.0127, 0.0001-0.0093, 0-0.0317 µg g^{-1} for aluminium, nickel, arsenic, cadmium, and lead, respectively. In this study, both aflatoxin and heavy metal levels in maize, barley and wheat samples examined as poultry feed ingredients were determined lower than in poultry feeds. According to these results, aflatoxin contamination in feeds is caused by the storage of feed in unsuitable conditions, and heavy metal contamination is largely due to the substances added to the feed later.

Keywords: aflatoxin, heavy metal, poultry feed, poultry feed ingredients

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INTRODUCTION

In Türkiye, chicken is one of the most important sources of animal protein for healthy and balanced nutrition. The production of layer chickens and broiler chickens has an important share in Türkiye's livestock production. The feeding of chickens is mostly based on mixed feed (Yucesoy, Kaya 2022). Toxins and heavy metals in feed can cause economic losses by leading to low productivity as well as a wide variety of effects and even death in animals (Kadakal et al. 2018). In addition, the consumption of such animal products can cause health problems in humans (Suleman et al. 2022).

For the development of animal breeding, it is very important to have sufficient information regarding mixed feed, and the hygienic quality of ingredients used in their production, which are becoming increasingly significant in animal nutrition. Recently, mycotoxins, caused by mold growth in many countries, and especially highly toxic aflatoxins (AFs), have become a major problem in the animal feeding and feed industry. Molds grow naturally in many agricultural products, especially red pepper, figs, nuts, corn, wheat, barley, rye, and many oilseeds as well as in the fields, gardens, after harvest, during the storage or processing of these products as food or animal feed. Mycotoxins are synthesized during mold growth (Ozkaynak et al. 2015). Mycotoxins are secondary metabolites that do not play an important role in normal metabolism but can cause acute and chronic effects, including carcinogenicity, mutagenicity, teratogenicity, and estrogenicity in humans and animals (Dimitrieska et al. 2016, Ismail et al. 2018, Gamal et al. 2023). They develop in almost all kinds of food and feed, and cause changes and deterioration in the quality and quantity of products by producing toxic compounds that are harmful to health (Liu et al. 2016, Kabak 2021, Belasli et al. 2023, Bhardwaj et al. 2023). AFB_1 is the most toxic AF. AFB_1 is the most common toxin with the highest rate of AF in naturally molded agricultural products, feed and processed food, and its most significant feature is its high carcinogenicity. AFG_1 and AFG_2 are less common than AFB_1, whereas AFB_2 is the least common mold AF. For this reason, because of its high toxicity, maximum levels have been determined only for AFB_1 in mixed feed (Aboagye-Nuamah et al. 2021). The upper tolerance limits for AFB_1, determined by the Turkish Food Codex (2011), the European Union (2002), and the Food and Agriculture Organization/World Health Organization (2002) in the poultry and in chick feed are 20 $\mu g\ kg^{-1}$ and 5 $\mu g\ kg^{-1}$, respectively.

AFs impact many animals, however, chicks, broilers, and ducklings, followed by turkey cubs, pheasants, chickens, and quails are the most susceptible (Allah Ditta et al. 2019). Aflatoxins cause developmental disorders, a decrease in egg yield and stagnation in chickens and chicks. Aflatoxicosis affects these animals chronically (Hossein, Gurbuz 2015). Both in humans and animals, the liver and kidney are very important for detoxification and execration of toxic elements, and they are thus also the most damaged organs due to an excess of toxic elements in feed or food. Moreover, AFs suppress the immune system in poultry, thereby making them more susceptible to many diseases (Eraslan et al. 2003, Dhanasekaran et al. 2009). Aflatoxins cause developmental disorders, a decrease in egg production and stagnation in chickens and chicks.

Heavy metals, as significant pollutants, are increasingly accumulating in the environment, thereby becoming an important source of environmental contamination. Metals such as lead (Pb), cadmium (Cd), aluminium (Al), nickel (Ni), and arsenic (As) known for their harmful effects on health are often referred to as "toxic heavy metals." These toxic heavy metals have no known function in the human body (Foulkes 2000). The entry of heavy metals due to environmental pollution into the food chain is significant in terms of animal and human health. In addition, metallic pollutants absorbed with water, nutrients, and air tend to accumulate in all living organisms (Selinus et al. 2005, Bakar et al. 2009). Contamination of poultry feed usually results from the use of plants contaminated with heavy metals in feed production (Ukpe et al. 2018). Metabolic disturbances induced by toxic effects in animals that receive mixed feed contaminated with heavy metals can lead to poor quality of animal products and poor socioeconomic effects achieved by producers. In addition, consumption of products from animals fed with contaminated feed may lead to undesirable effects on humans (Santhi et al. 2008). The main undesirable effects include increased sensitivity to certain diseases and other stressors, cancer, premature ageing, neural symptoms, weak bone formation, pain in the muscles, anorexia, anaemia, premature death, and birth anomalies (Aljohani 2023).

Data on AF_s and heavy metal levels in the poultry feed in Türkiye are limited. Therefore, this study aimed to examine the accumulation of AFs and toxic heavy metals in poultry feed and feed ingredients from Van, Türkiye.

MATERIALS AND METHODS

Chemicals

Aflatoxin standards (AFB_1, AFB_2, AFG_1 and AFG_2) were supplied by Sigma-Aldrich (St Louis, MO, USA). All solvents of the mobile phase were HPLC grade and purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q Plus apparatus from Millipore (Milford, MA, USA). Phosphate-buffered saline (PBS) was prepared by dissolving Sigma-Aldrich PBS tablets in distilled water. The immunoaffinity columns for aflatoxins (product code P07) were purchased from R-biopharma (R-Biopharma Rhone Ltd, Scotland, UK). Analytical grade nitric acid, perhydrochloric acid (Merck, Germany) and deionised water (Milli-Q System, Millipore, Billerica, USA) were used for the preparation of all solutions. All plastic and glassware were soaked in 10% (v/v) HNO_3 overnight and rinsed with deionised water before use for heavy metal analysis. Multielement ICP-MS calibration standard (Inorganic Ventures, USA) was used as a standard solution for Al, Ni, As, Cd and Pb.

Sample collection

The study included a total of 30 feed samples consisting of 15 feed ingredients and 15 mixed feeds, sold as poultry feed in the Van province. Samples were obtained from factories that produce poultry feed, chicken farms, and feed stores in the Van province. The samples placed in sterile bags were transported to the laboratory promptly so that they would not be exposed to air or high temperatures. The feed samples were milled and sieved, and 100 g samples were taken from the milled feed and kept in the refrigerator (4°C) until AF extraction and heavy metal analysis. It would have been interesting to collect different types of feed samples (i.e. chicken starter, grower, finisher feed, broiler starter, grower, finisher feed and layer starter, grower, finisher feed) for analysis.

Aflatoxin analyses

AF assays were performed on a Shimadzu Prominence high-performance liquid chromatographer (HPLC). Finely milled feed samples (50 g) were weighed and added into 5 g NaCl + 100 mL (80% methanol) solution and mixed in a stirrer for 3 minutes. The mixture was then filtered through Whatman 4 filter paper. Then, 2 mL of the filtered sample was diluted with 14 mL PBS and passed through the immunoaffinity column containing AFB_1, AFB_2, AFG_1, and AFG_2 antigens. The column was washed with 20 mL of PBS. Finally, the column was washed with 1 mL methanol and 1 mL deionized distilled water, and this final mixture was transferred to a tube and vortexed. The final solution was transferred to a 1.5 mL HPLC vial, and 100 μL of the solution was injected into the HPLC system with increased sensitivity by Cobra Cell (set to 100 μA). The samples were analyzed sequentially using a Fluorescence Detector (set to 1 mL min^{-1} mobile phase and exc: 362 nm, suction: 425 nm) containing methanol/acetonitrile/water (220 mL; 160 mL; 620 mL) added with 350 μL 4M nitric acid and 119 mg potassium bromide using the Spherisorb ODS-3, 250 × 4.6 mm, 5 μm, HPLC column (AOAC 2000). The multipoint calibration solutions were prepared and calibrated by drawing standard curves by the AOAC method 999.07 using the HPLC device (Supelco Inc., Bellefonte, PA) and an AF (AFB_1, AFB_2, AFG_1, and AFG_2) mixed standard kit. The peaks in the chromatogram of each extracted sample were compared with the retention time of the standard peak. When the sample was confirmed to contain aflatoxins, the amounts of AFB1, AFB2, AFG1, and AFG2 in the injected aliquot were calculated based on the corresponding standard calibration curve. Concentrations below the device's detection limit (LOD) were considered undetectable and denoted as "UDL" in the tables.

Heavy metal analyses

The collected samples were ground and weighed 0.2 g, 7 mL of HNO_3 and 1 mL of H_2O_2 (65%; Merck, Darmstadt, Germany), while H_2O_2 was 30% v/v (Merck, Darmstadt, Germany) were added to the weighed samples. Samples were solubilized at the appropriate setting of a Milestone ETHOS Easy microwave system according to Milestone's procedure (2011) at 200°C for 15 min. The microwave is preferred because it accelerates the burning process and minimizes the possible contamination during the process (Usero et al. 2004). The tubes, which were taken out of the oven after the burning procedure, were cooled down at room temperature and the solution in the tubes was poured into 25 mL polypropylene volumetric flasks. The amount of the solution in the volumetric flasks was completed to 25 mL with distilled water. A blank digest was carried out in the same way. The toxic metal content analysis of the samples was performed with an ICP-MS device (Thermo Scientific UK, X Series 2). For each of the 30 samples, three parallel samples were analyzed and the final results were expressed by taking the average of the results of three measurements. Calibrations were carried out using a multi-element ICP-MS Standard provided by Inorganic Ventures (Christiansburg, VA, USA) with Al, Ni, As, Cd and Pb concentrations of 142 μg L^{-1}, 62 μg L^{-1}, 60 μg L^{-1}, 7 μg L^{-1} and 20 μg L^{-1}, respectively. Calibration curves were prepared for each element and showed good linearity within the analytical range. The limits of detection (LODs), defined as the concentration of each element corresponding to three times the standard deviation of 10 reagent blanks, were 0.013, 0.00051, 0.000033, 0.0003 and 0.00006 μg kg^{-1} for Al, Ni, As, Cd and Pb respectively. The limits of quantification (LOQs), defined as the concentration of each element corresponding to 10 times the standard deviation of 10 reagent blanks, were 0.043, 0.0168, 0.00011, 0.00099 and 0.00020 μg kg^{-1} for Al, Ni, As, Cd and Pb respectively. All the spiked samples were digested following the procedure described earlier in triplicates. Procedural blanks were analyzed together with the samples to monitor potential contamination during digestion and analysis. The relative standard deviations (RSDs) of replicates were less than 10% (Ademse et al. 2009).

Statistical analyses

Aflatoxin concentrations were presented descriptively for individual samples because the number of positive samples was limited. Therefore, no statistical comparison was performed for aflatoxin data. In contrast, heavy metal concentrations were statistically analyzed because measurable levels were detected in all samples. Descriptive statistics for the continuous variables (characteristics) were presented as mean, standard deviation, median, IQR, minimum and maximum values. The Kolmogorov Simirnov test was used for normality test for continuous variables. After the normality test, one-way ANOVA was performed to compare the means in normally distributed characteristics. However, for the non-normally distributed characteristics, the Kruskal-Wallis test was performed. Following these analyses, the Duncan and Dunn multiple comparison tests were used to determine different groups in normal and non-normal distributed characteristics, respectively. Statistically significance level was considered as 5% and SPSS (ver: 25) statistical program was used for all statistical computations.

RESULTS AND DISCUSSION

The present study evaluated the occurrence of aflatoxins and selected toxic heavy metals in poultry feed and feed ingredients collected from the Van province, Türkiye. Feed contamination with mycotoxins and toxic metals is an important concern for both animal health and food safety because contaminated feed may lead to reduced productivity, immunosuppression, and the transfer of contaminants into animal-derived food products (Bryden 2012).

AFB_1, AFB_2, AFG_1, and AFG_2 levels (μg kg^{-1}) detected in the feed samples obtained from the factories that produce poultry feed, chicken farms, and feed stores in the Van province are presented in Table 1, and the toxic heavy metal levels are presented in Table 2.

In the present study, AF was not detected in samples of corn, barley, and wheat which are used as feed ingredients (Table 1). AFB_1 and AFB_2 were detected in two of the chick grower feed samples; AFB_1, AFB_2, AFG_1, and AFG_2 in one of the broiler chicken feed samples; AFB_1 and AFB_2 in one and only AFB_2 in one of the broiler chicken feed samples; and AFB_2 in one, AFB_1 and AFB_2 in two, and AFB_1, AFB_2, and AFG_1 in one of the layer chicken feed samples. In the total 30 feed samples analyzed, 23% had 4.631-50.917 μg kg^{-1} of AFB_1, 30% had 2.030-55.238 μg kg^{-1} of AFB_2, 7% had 1.645-112.013 μg kg^{-1} of AFG_1, and 3% had 0.055 μg kg^{-1} of AFG_2. The total AF (B_1, B_2, G_1, and G_2) levels were between 2.131-171.937 μg kg^{-1} in 9 samples (30%) with AF (Table 3). In the present study, 12% of positive samples contained higher AF levels than the legal limit for AF stipulated in many countries.

Although AF analysis in poultry feed from the Van province revealed no AF in corn, barley, and wheat used as feed ingredients, varied AF levels were found in the mixed feed (Table 1). Rüstemoglu and Karadas (2016) also detected 4.74 μg kg^{-1} AFB1, 4.27 μg kg^{-1} AFB_2 and 0.38 AFG_2 in only one sample taken from one factory in June among the chick feed samples they collected in April, May and June from 5 factories producing feed in the Van region. In this study, AF was detected in 9 of the mixed feed samples, and considering the acceptable upper limits (20 μg kg^{-1} for adult and 5 μg kg^{-1} for non-adult), AFB_1 was detected above the limit in one of the broiler chicken

Table 1

Aflatoxin levels determined in poultry feed ingredients and poultry feed samples

Sample No		Aflatoxin levels (μg kg^{-1})				
		B_1	B_2	G_1	G_2	total aflatoxin level
Poultry feed ingredients	maize	UDL	UDL	UDL	UDL	UDL
	maize	UDL	UDL	UDL	UDL	UDL
	maize	UDL	UDL	UDL	UDL	UDL
	maize	UDL	UDL	UDL	UDL	UDL
	maize	UDL	UDL	UDL	UDL	UDL
	wheat	UDL	UDL	UDL	UDL	UDL
	wheat	UDL	UDL	UDL	UDL	UDL
	wheat	UDL	UDL	UDL	UDL	UDL
	wheat	UDL	UDL	UDL	UDL	UDL
	wheat	UDL	UDL	UDL	UDL	UDL
	barley	UDL	UDL	UDL	UDL	UDL
	barley	UDL	UDL	UDL	UDL	UDL
	barley	UDL	UDL	UDL	UDL	UDL
	barley	UDL	UDL	UDL	UDL	UDL
	barley	UDL	UDL	UDL	UDL	UDL
Poultry feed	chick grower feed	UDL	UDL	UDL	UDL	UDL
	chick grower feed	11.548	2.244	UDL	UDL	13.792
	chick grower feed	8.783	2.030	UDL	UDL	10.813
	chick grower feed	UDL	UDL	UDL	UDL	UDL
	chick grower feed	UDL	UDL	UDL	UDL	UDL
	broiler chicken feed	UDL	UDL	UDL	UDL	UDL
	broiler chicken feed	4.631	55.238	112.013	0.055	171.937
	broiler chicken feed	UDL	2.183	UDL	UDL	2.183
	broiler chicken feed	29.753	2.846	UDL	UDL	32.599
	broiler chicken feed	UDL	UDL	UDL	UDL	UDL
	layer chicken feed	50.917	3.373	UDL	UDL	54.29
	layer chicken feed	9.437	2.377	1.645	UDL	13.459
	layer chicken feed	UDL	2.130	UDL	UDL	2.130
	layer chicken feed	10.951	2.212	UDL	UDL	13.163
	layer chicken feed	UDL	UDL	UDL	UDL	UDL
Maximum aflatoxin level that can be found in foods (EU 2002, FAO/WHO 2002, TGK 2014)		adult: 20 chick: 5	–	–	–	–

UDL – undetectable level indicates that the concentration was below the limit of detection (LOD)

Table 2

Toxic heavy metal levels determined in poultry feed ingredients and poultry feed samples (μg g^{-1})

	Group	n	Mean ± SD	Median	IQR	Max.	Min	p
Al	maize	5	2.53 ± 0.750^{c}	3.02100	1.33350	3.12100	1.45400	0.001
	barley	5	8.92 ± 3.510^{b}	8.92050	5.79801	13.79800	4.04300	
	wheat	5	3.84 ± 2.694^{c}	4.00600	5.04503	7.64100	1.09700	
	chick grower feed	5	15.78 ± 0.783^{a}	15.5000	1.48772	16.83850	15.00000	
	brolier chicken feed	5	11.08 ± 4.545^{ab}	10.9310	7.80751	17.30100	4.84300	
	layer chicken feed	5	11.69 ± 6.396^{ab}	8.61200	12.2925	19.04600	5.90300	
Ni	maize	5	0.052 ± 0.019^{c}	0.05600	0.03441	0.07400	0.02600	0.001
	barley	5	0.064 ± 0.014^{c}	0.06500	0.02632	0.08200	0.04600	
	wheat	5	0.068 ± 0.045^{c}	0.06000	0.08010	0.13900	0.02600	
	chick grower feed	5	0.370 ± 0.039^{a}	0.36000	0.07701	0.41200	0.32800	
	brolier chicken feed	5	0.218 ± 0.060^{b}	0.18000	0.11111	0.28800	0.16900	
	layer chicken feed	5	0.287 ± 0.130^{ab}	0.29500	0.23523	0.47800	0.14800	
As	maize	5	0.0014 ± 0.00064^{c}	0.00160	0.00121	0.00210	0.00050	0.001
	barley	5	0.0035 ± 0.00055^{bc}	0.00350	0.00112	0.00410	0.00290	
	wheat	5	0.0032 ± 0.00194^{bc}	0.00280	0.00301	0.00650	0.00160	
	chick grower feed	5	0.0090 ± 0.00221^{a}	0.00900	0.00442	0.01140	0.00660	
	brolier chicken feed	5	0.0050 ± 0.00082^{b}	0.00440	0.00153	0.00600	0.00440	
	layer chicken feed	5	0.0076 ± 0.00298^{a}	0.00680	0.00451	0.01270	0.00500	

cont Table 2

	Group	n	Mean ± SD	Median	IQR	Max.	Min	p
Cd	maize	5	0.0006 ± 0.00035^{c}	0.00060	0.00065	0.00100	0.00010	0.003
	barley	5	0.0003 ± 0.00005^{c}	0.00025	0.00010	0.00030	0.00020	
	wheat	5	0.0024 ± 0.00384^{c}	0.00070	0.00455	0.00930	0.00060	
	chick grower feed	5	0.0052 ± 0.00158^{a}	0.00520	0.00277	0.00730	0.00300	
	brolier chicken feed	5	0.0023 ± 0.00108^{c}	0.00200	0.00195	0.00400	0.00140	
	layer chicken feed	5	0.0038 ± 0.00157^{ab}	0.00350	0.00265	0.00640	0.00240	
Pb	maize	5	$0.0007 \pm 0.00093^{\mathrm{d}}$	0.00020	0.00165	0.00210	0.00000	0.001
	barley	5	$0.0008 \pm 0.00010^{\mathrm{d}}$	0.00080	0.00020	0.00090	0.00070	
	wheat	5	0.0055 ± 0.00815^{c}	0.00040	0.01320	0.01880	0.00000	
	chick grower feed	5	0.0260 ± 0.00436^{a}	0.02550	0.00792	0.03170	0.02020	
	brolier chicken feed	5	0.0054 ± 0.00194^{c}	0.00620	0.00370	0.00740	0.00300	
	layer chicken feed	5	0.0124 ± 0.00966^{b}	0.01120	0.01740	0.02720	0.00310	

n – number of samples analyzed in each group, a, b, c – different lowercase letters for each characteristic represent statistically significant differences between the groups

Table 3

Occurrence of aflatoxin contamination in poultry feed samples

Feed type	Number of samples	Positive samples	Contamination rate (%)	Total AF range (μg kg^{-1})
Feed ingredients	15	0	0	UDL
Chick grower feed	5	2	40	10.813-13.792
Broiler feed	5	3	60	2.183-171.937
Layer feed	5	4	80	2.130-54.29
Total	30	9	30	2.130-171.937

UDL – undetectable level indicates that the concentration was below the limit of detection (LOD)

feed, one of the layer chicken feed, and two of the chick grower feed. Similar to our results, the mixed feed has been reported to be more contaminated than cereals in animal feed (625 feed and 1120 feed ingredients) in Poland (Juszkiewicz 1992). Anjum et al. (2012) found that the maximum AFB_1 levels (mean: 56 μg kg^{-1}) in 77 poultry feed ingredients were higher than AFB_1 levels (78 μg kg^{-1}) in 410 poultry feed. Nemati et al. (2014) found a low AFB_1 level in poultry feed ingredients containing wheat, wheat bran, and corn (1.54, 3.05, and 2.35 μg kg^{-1}, respectively). Ghaemmaghami et al. (2024) found that the AFB1 level in pelleted and mash feed was higher than the AFB1 level of poultry feed ingredients such as corn and soybean meal in Iran, and reported that finished feed, especially in pellet form, had a higher risk of aflatoxin contamination compared to ingredients in poultry feed. In contrast, Yazdanpanah et al. (2001) found that 89% of corn samples were contaminated at an average level of 15.83 μg kg^{-1} in AFB_1 analysis on 14 barley samples and 9 corn samples in the Golestan and Mazandaran provinces in northern Iran. Anjum et al. (2011) reported high AFB_1 levels in the commercial poultry feed ingredients collected in Pakistan. Fareed et al. (2014) found that the total AF level was lower in the feed compared to the feed ingredients in the analysis performed on 114 poultry feed material samples and 72 feed samples. Maqbool et al. (2009) reported that feed ingredients have been more contaminated with AF compared to the feed.

The occurrence of mycotoxins in feed ingredients depends on several factors that include climatic conditions, diversity of fungi contaminating the crops, harvesting methods of the individual crops, storage practices, and seasonal variations, while the types and levels of mycotoxins in the feed largely depend on the mycotoxins in the individual feed ingredients, the mix/proportion of feed ingredients, feed processing techniques, and storage practices (Warth, Parich 2012, Ezekiel et al. 2014). Therefore, the reason for higher levels of aflatoxin detection in these feeds may be that these feeds were stored under inappropriate temperature and humidity conditions. Saleemullah et al. (2006) and Anjum et al. (2012) studied the effect of storage conditions on total aflatoxin yield and found a positive relationship between the total aflatoxin concentration and moisture content.

Food and feed products can be contaminated at any stage of production. In particular, mycotoxins that are harmful to human and animal health can be formed during the harvesting of plants, during transportation and storage, during the preparation of food or feed and technological processes involved, when hot and humid environments suitable for the development of molds are formed (Tonbak, Demir 2012, Udomkun et al. 2017).

In the present study, the highest AFB_1 levels (9.437-50.917 μg kg^{-1}) were found in the layer chicken feed. Similarly, in a study conducted in Brazil, the mean AF level in 88.2% of 34 broiler feed samples was 10.48 μg kg^{-1} and 8.41 μg kg^{-1} by enzyme-linked immunosorbent assay (ELISA) and HPLC, respectively. In the same study, AF contamination was 20.83 and 19.75 μg kg^{-1} by ELISA and HPLC, respectively, in 92% (n=36) of layer chicken feed samples (Rossi et al. 2012). The study by Şanli et al. (1982) on 96 total feed samples (poultry feed and feed ingredients) using thin-layer chromatography found AFB_1 at a level of 9.30 ± 2.67 μg kg^{-1} in the layer chicken feed samples, 4.02 ± 1.11 μg kg^{-1} in the broiler chicken feed samples, and 6.04 ± 1.56 μg kg^{-1} in the feed ingredients.

In addition to mycotoxins, heavy metal contamination in animal feed has also become a major environmental and food safety concern. Toxic metals such as cadmium, lead, arsenic, and nickel may enter feed materials through contaminated soil, irrigation water, fertilizers, or industrial emissions (Santos et al. 2010). Once ingested by animals, these metals can accumulate in tissues such as the liver, kidney, and muscle, potentially posing a risk to human consumers through the food chain (Musa 2012, Chang et al. 2015, WHO 2020, Kim 2023).

Significant differences were observed among feed groups for all analyzed metals (p<0.05). In the heavy metal analysis, lead was not detected in only 2 corn samples and 1 wheat samples, while certain amounts of Al (2.53-15.78 μg g^{-1}), Ni (0.052-0.370 μg g^{-1}), As (0.0014-0.0090 μg g^{-1}), Cd (0.0003-0.0052 μg g^{-1}), and Pb (0.0007-0.026 μg g^{-1}) were detected in all other samples (Table 2). The highest aluminum, nickel, arsenic, cadmium, and lead concentrations were generally detected in compound poultry feeds, particularly chick grower feed. Wheat samples contained the lowest Al and Cd levels, while corn samples had the lowest Ni, As, and Pb levels.

The heavy metal concentrations detected in the analyzed feed samples were evaluated in relation to international regulatory limits for feed safety (Table 4). Al, Ni, As, Cd and Pb levels detected in the present study were below the maximum tolerable trace element levels for poultry (NRC 2005, Sugaya et al. 2016), and the maximum levels found in foods (EU 2002, FAO/WHO 2002).

Of all heavy metals analyzed, aluminium was determined in the highest amounts in the poultry feed ingredients and poultry feed sampled in the Van province (Table 2, Figure 1). The order of heavy metal levels detected in the

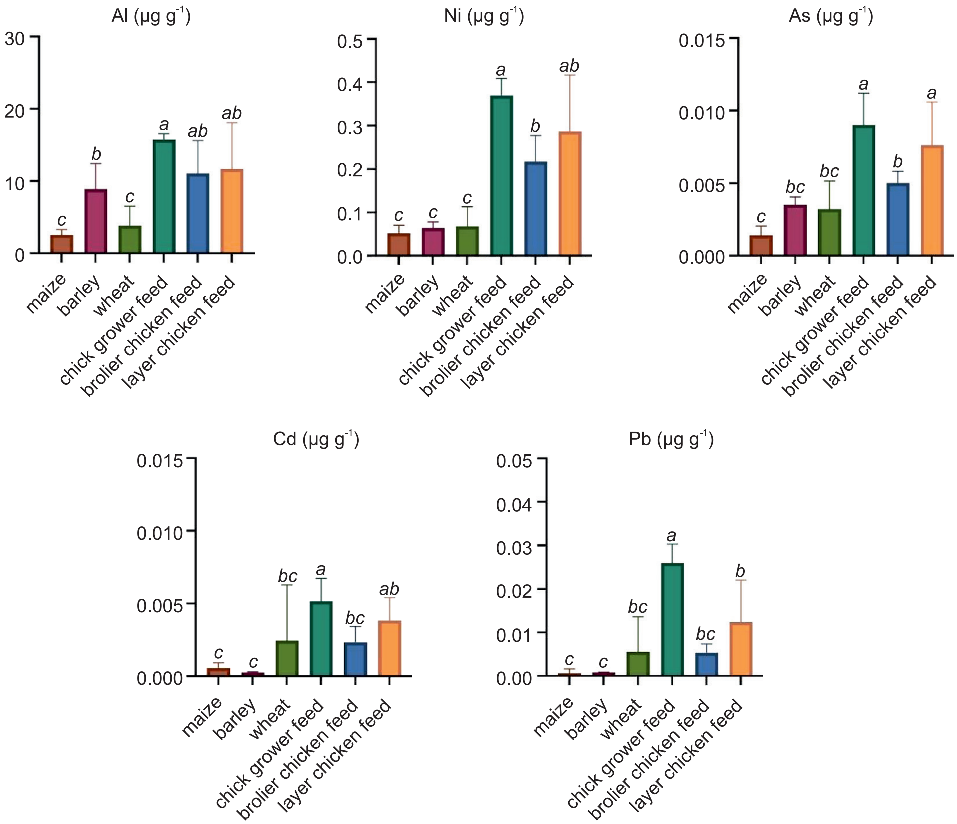

Fig. 1. Mean concentrations of Al, Ni, As, Cd, and Pb in all samples

Table 4

Maximum permissible limits reported by international organizations and literature

International organization or literature	Toxic heavy metals limits				
	Al	Ni	As	Cd	Pb
NRC (2005)	-	250	30	10	10
Suganya et al. (2016)	200	300	100	0.5	30
EU (2002)	-	-	2	0.5	5
EU (2002), FAO/WHO (2002)	-	-	1	500	-

feed ingredients and poultry feed were Al > Ni > Pb > As > Cd. The comparison of the poultry feed and feed ingredients revealed that the heavy metal levels in the poultry feed were relatively higher than those detected in the feed ingredients. This suggests that heavy metal contamination may originate from mineral premixes, additives, or other components incorporated during feed formulation. The mixed feed sampled for the present study contained soybean flour, animal by-products, oils, and premixes of vitamins and minerals in addition to corn, barley, and wheat. Because of the heavy metal load in these feed ingredients, the final feed products may have been contaminated. Even if the heavy metal levels in the feed did not exceed the specified limits, because of the continuous mixing of ingredients polluted with heavy metals in the feed consumed by poultry, health problems may arise in consumers of such poultry meat and eggs. Therefore, it is important to constantly monitor heavy metal concentrations to limit their negative effects on public health (European Commission 2002, 2003). Although the contamination of feed by toxic metals may not be completely prevented, this type of pollution should be reduced to lower overall feed contamination.

Results of previous studies on heavy metal analysis of poultry feeds are given in Table 5.

Table 5

Comparison of our results with other studies that examine heavy metal levels in poultry feeds ($\mu g\ g^{-1}$)

Literature and sample location	Al	Ni	As	Cd	Pb
This study, Türkiye (Van)	1. 1.097-19.046	0.026-0.478	0.0005-0.0127	0.0001-0.0093	0-0.0317
İslam et al. (2007), Bangladesh	ND	0.013-5.163	0.0069-0.764	0.0232-0.1852	0.602-20.65
Alkhalaf et al. (2010), Nijerya	ND	0.45-3.26	ND	0.055-0.249	0 0.15-3.21
Okoye et al. (2011), Nijerya	ND	2 2.250-4.875	ND	0 0.038-0.463	1.10-7.85
Zhang et al. (2012), northeast China	ND	ND	0.12-1.22	0.00-1.26	ND
Rashid et al. (2012), Bangladesh (Dhaka)	ND	ND	0.006	ND	0.69-0.86
Bukar and Said (2014), Nijerya (Kano metropolis)	ND	1.03-2.06	ND	0.53-3.19	0.27-0.80
Imran et al. (2014), Pakistan (Kasur)	ND	2.91-5.52	2.37 - 0.76	1.41 - 0.11	2.33-7.90
Iqbal (2023), Pakistan (Rawalpindi and Islam-abad)	ND	ND	ND	UDL	0.93-2.32
Keutchatang et al. (2023), Cameroon (Centre, Littoral, and western regions)	ND	7.146- 22.575	0.002-2.819	0.024-0.033	0.075-0.996

UDL – undetectable level, ND – not done

According to Table 5, Ni, As, Cd, and Pb levels detected in poultry feed (Islam et al. 2007, Alkhalaf et al. 2010, Okoye et al. 2011, Zhang et al. 2012, Bukar and Said 2014, Rashid et al. 2012, Imran et al. 2014, Keutchatang et al. 2023) in previous studies were significantly higher than those detected in the present study. Comparison with previous studies indicate that contamination levels may vary considerably depending on the geographic region, environmental conditions, and feed management practices. Environmental factors such as temperature, humidity, and storage duration are known to influence aflatoxin formation, whereas heavy metal contamination is often associated with environmental pollution and agricultural practices (Santos et al. 2010).

CONCLUSIONS

In conclusion, the present study found that AF and heavy metal concentrations in corn, barley, and wheat samples, analyzed as feed ingredients, were lower than those of mixed feed samples. Special measures should be taken to prevent AF contamination, especially since AF levels in some feed were found to be above the specified limits. Therefore, it is recommended to assess any health risk arising from exposure to toxins, and regularly examine AF and heavy metals in foods that could harm the end-consumer's health.

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Author contributions

U.M.Y., Y.K. – plan, design, U.M.Y., Y.K. – material, methods and data collection, U.M.Y. – data analysis and comments, U.M.Y. – writing and proofreading. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The author declares no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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