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Mycotoxins and Heavy Metals of Poultry Feeds from the Centre, Littoral, and Western Regions of Cameroon

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ABSTRACT

Heavy metals are a group of elements that could be found in poultry feeds and influence poultry production. Poultry feed generally consists of agricultural products, such as maize, groundnuts, and wheat, which may also be contaminated by mycotoxins. The use of mycotoxins and heavy metals contaminated feed in the poultry sector might represent a potential source of cross-contamination for humans. This study aimed to analyze total aflatoxins (AFs), aflatoxin B_1 (AFB_1), ochratoxin A (OTA), chromium, copper, nickel, zinc, arsenic, cadmium, lead, and mercury in poultry feed from the Centre, Littoral, and Western regions of Cameroon. In this order, six local broiler feeds, six local layer feeds, and three imported layer feeds were randomly collected from each region and analyzed using inductively coupled plasma spectrometry for heavy metals and competitive indirect ELISA for mycotoxins. The results indicated that all feed samples contained the analyzed mycotoxins and heavy metals. The ranges for the mean concentrations of mycotoxins were 3.5-19.7, 2.7-19.3, 0.8-1.1 µg/kg for AFs, AFB_1, and OTA, respectively. They were globally below the established regulated limits (20 µg/kg for AFs, 10 µg/kg for AFB_1 and 5 µg/kg for OTA). The bulk layer feed from the Littoral region had the highest lead (995.8 ± 0.4 µg/kg) and cadmium (3.3 ± 0.0 µg/kg) concentrations. The average concentration of lead was above the permissible limit (10 µg/kg). Bulk broiler feed from the Littoral region scored the highest concentration of arsenic (2819.4 ± 0.1 µg/kg) above the permissible limit (500 µg/kg). Bulk broiler feed from the Centre region showed the highest concentration of mercury (5.6 ± 0.0 µg/kg) although lower than the permissible limit of 100 µg/kg. This study demonstrates that there are potential safety issues associated to poultry feeds used in some regions of Cameroon. It suggests a possible low productivity of poultry and health issues for consumers.

Keywords: Aflatoxin, feed, Contamination, Heavy metals, Ochratoxin A, Poultry

INTRODUCTION

The poultry sector is known as an important source of protein and other useful nutrients for human nutrition and health in Cameroon (Guetiya Wadoum et al., 2016). Chickens are easy to rear (Paryad and Mahmoudi, 2008), available at low prices, and known as food for billions of people, including those who live in low-middle-income countries (Aral et al., 2013), such as Cameroon. Poultry production represents 42% of meat production. In Cameroon, chicken meat and eggs consumption increased from 2.2 kg to 5.6 kg and 16 to 52 eggs between 2006 and 2016, respectively. In addition, poultry represents 4% of the gross domestic product (Höffler, 2018). Poultry feeds are generally a mixture of agricultural products, such as maize, groundnuts, and wheat, which may be contaminated by mycotoxins and heavy metals (Abia et al., 2013a, Akinmusire et al., 2018). The use of such contaminated feed in the poultry sector may result in poultry productivity and in a source of human contamination. It is recommended to initially analyze the different contaminants found in these feeds to limit the risks of feed quality on the poultry productivity directly and indirectly on human health. However, studies on poultry feed contamination are scarce, and it refers to 2013 in Cameroon. Mycotoxins are secondary metabolites produced by three genera (*Aspergillus*, *Penicillium,* and *Fusarium*) of fungi which can produce more than 500 toxins. Among these mycotoxins, Aflatoxins and Ochratoxin A (OTA), exhibit pathogenic characteristics (Becer and Filazi, 2010; Kaya, 2014). Toxic heavy metals are mineral elements with a specific weight greater than $5g/cm^3$ (Demirezen and Uruc, 2006). These mineral elements are a serious concern due to their impacts (toxicity, bioaccumulation, and biomagnifications) in the food chain (Demirezen and Uruc, 2006; Hazrat et al., 2019). Considering the fact that contamination of poultry feed by contaminants, such as mycotoxins and toxic metals, cannot be entirely avoided due to favorable climatic conditions for their development (Tatfo Keutchatang et al., 2021) and the availability of pollutants in the environment, there is a need for such contamination to be minimized, and to reduce theirs side effects on animal and human by one health approach (WHO, 2017). This study was initiated to enrich the data already available on the contamination of poultry feed by mycotoxins and heavy metals in Cameroon. It aimed to analyze mycotoxins (total aflatoxins, aflatoxin B_1, and OTA) as well as eight heavy metals in poultry feeds collected in the Centre, Littoral, and West regions using enzyme-linked immunosorbent assay (ELISA) and inductively coupled plasma spectrometry (ICP- OES), respectively.

MATERIALS AND METHODS

Study area

The current study was conducted on poultry farms located in three regions of Cameroon: Centre, Littoral, and West. These regions are the large areas of production and consumption of chickens and eggs (Teleu and Ngatchou, 2006). The study was conducted from January to December 2019.

Sampling design

A total number of 15 samples of chicken feed, constituted of 6 local broiler feed (2 per region), 6 local layer feed (2 per region), and 3 imported layer feed (1 per region) eaten by broiler and layer chickens were collected from poultry farms. Local feed samples were collected from layer and broiler farms, while imported layer feeds were collected from imported feed outlets. Indeed, a preliminary study reported the classification of chicken farms in these three regions into two groups (moderate and high risk of biosecurity) according to biosecurity score (Tatfo Keutchatang et al., 2021). Feed sampling was done as described by the European Commission (2006) Directive No. 401/2006 (EC, 2006). In each selected farm or outlet, 4 kg of feed was sampled. Different points of four randomly selected feeds (50 Kg bags) from those available were duplicated. A total of four bags randomly selected for feed sampling were chosen from the same strip to reduce variability and ensure the effective representativeness of the strip. Each sub-sample of 1 kg consisted of three portions of 300 to 350 g. The sub-samples were collected manually using a probe at three points top, middle, and bottom of feed bags. The feeds taken from each point were homogenized in bags, and 1/4 of each was collected to provide 15 representative samples as 4 feed samples in the Centre, 4 in the Littoral, 4 in the West, and 3 outlets. The samples were conditioned in polystyrene bags and transported to the laboratory for quality control at the Centre for Food and Nutrition Research of the IMPM, Yaoundé, Cameroon, where the feed samples were ground with a blender (Black & Decker®, England), weighed in several aliquots of 5g using a scale (Mettler Toledo, USA), and stored in sterile plastic bags at -20°C for analysis. The samples were kept in the laboratory for a maximum of 7 days.

Sample preparation and analysis

Water content of different samples

Water content was determined using the reference methods of the Association of Official Analytical Chemists (AOAC, 2000) for bulk chicken feeds. An amount of 5 g of each sample in triplicate was dried at 105°C (Rolabo, Germany) until constant weight in an aluminum foil previously dried and weighed the dried samples were cooled in a desiccator (Borosilicate Glass 3.3, Indane Chemical Company, Borivali, Mumbai, Maharashtra) for 30 minutes and reweighed. The water content of each sample was determined by calculating the differences between the masses of the fresh and dried samples (AOAC, 2000).

Determination of mycotoxin content

Total Aflatoxins (AFs), Aflatoxin B_1 (AFB_1), and OTA concentrations in the samples were determined using quantitative enzyme-linked immune sorbent assay kits (ELISA, BIOO Scientific Corporation, MaxSignal®, USA). Samples containing 2 g of ground bulk chicken feed were mixed with 25 mL of 70% methanol (HPLC grade, Merck, Germany) in 50 mL falcon tubes for 10 minutes using a vortex, centrifuged at 4000 × g for 10 minutes using the Rotofix 32 A, centrifuge (Germany). Then, 100 µL of the supernatant was collected and diluted with 700 µL 70% methanol (HPLC grade, Merck, Germany). The mixture was used for total AFs, AFB_1, and OTA analyses following the kit manufacturer's instructions and as described by Tatfo Keutchatang et al. (2022). The concentrations of determined mycotoxins were inversely proportional to the color intensity established using an automated microplate reader (EL × 800, BIOTEK, Instruments Inc., Winooski, VT, United States) at 450 nm and estimated based on a calibration curve.

Determination of heavy metal content

Sample preparation

The dried samples were cooled in a desiccator for 30 minutes and reweighed. The different bulk samples were dried and ground with a blender (Black & Decker®, England), then weighed in several aliquots of 500 mg by using a scale (Mettler Tolero, USA). Then, 500 mg of each powder bulk sample and 50 mL of nitric acid were introduced into the container to obtain a mixture left to stand overnight (Broekaert, 2005).

Inductively coupled plasma with optical emission spectrometry

The analysis was conducted as described by Broekaert (2005). The selected heavy metals contained Arsenic (As), Copper (Cu), Cadmium (Cd), Chromium (Cr), Mercury (Hg), Nickel (Ni), Lead (Pb), and Zinc (Zn) contents were determined. These metals were selected based on their benefits and toxicity in living organisms. The detection of the elements present in the analyte was performed by emission. The nebulized analyte was driven by a peristaltic pump to obtain an aerosol that was transported in the plasma, where it was desolvated, vaporized, atomized, or ionized. The return to a lower energy state was accompanied by the emission of radiation characteristic of the elements. A monochromator separated the different wavelengths. The wavelengths of the analyzed elements and the preparation of their standards are presented in Table 1.

Table 1. Standard solution used during the determination of metals

	Volumes (mL)						
Solutions étalons	1	2	3	4	5	6	7
Solutions Cd, Ni, Pb, Cr, As, Hg, Zn, Cu	125	150	175	200	2 25	250	–
HNO_3 conc.	125	100	75	50	25	–	250
Volume final	250	250	250	250	250	250	

Cd: Cadmium, Ni: Nickel, Pb: Lead, Cr: Chromium, As: Arsenic, Hg: Mercury, Zn: Zinc, Cu: Copper, HNO_3 Conc: Concentrated nitric acid

Mineralization for heavy metals determination

An amount of 500 mg of sample was weighed and introduced for digestion in a DigiTUBE containing a mixture of 5 mL of nitric acid and 10 mL concentrated hydrogen peroxide for 16 hours at 25°C. Then, the mixture was brought to 95°C for 2 hours in a graphite heating block before being filtered. In each series of tubes at least three blanks were placed and three controls prepared. After the installation of the tracks on DigiPREP, the tubes were rotated. For this purpose, the locating lugs matched the notches and the bottom of the tubes was in contact with the bottom of the graphite block. The blanks were covered with perforated plugs to be able to insert the DigiPROBE temperature probe inside. The probe was placed low enough to be immersed in the liquid without touching the bottom of the tube. The temperature controller was switched on and the temperature program was selected. After allowing the tubes to cool to 25°C, the volume of each sample was adjusted to 20 mL (Broekaert, 2005).

Quality control

The analytical test for mycotoxins was conducted using the internal quality control (IQC) approach and validated before usage. The quality control was performed by choosing five different IQCs as follows, calibration, blanks, mid-range standard, spiked standard solution, certified references material, and duplicates. Results were discarded and the sample was if a sample did ~~was~~ not met the acceptance criteria, and the sample was reanalysed. The limit of detection (LOD) of the analysed samples was within the range of 0.06-0.3 µg/kg for AFs and 0.3-0.6 µg/kg for OTA, while the limit of quantification (LOQ) was in the range of 0.2-1 µg/kg for AFs and 1-2 µg/kg for OTA. Samples with values below LOQ were recorded as non-detectable (CEAEQ, 2015). The calibration standards for metals were prepared from certified standards. A total number of four external reference samples and one standard reference sample from the National Institute of Standards and Technology (NIST) were introduced into each series for analysis.

Statistical analysis

Data obtained were transferred into Microsoft Excel for the calculation of the concentrations of µg kg−1. The obtained data were then subjected to analysis of variance (ANOVA) and Student's T test for paired samples at the significance level of 5% for means comparison using a statistical package, SPSS version 20.0 for windows. Results were expressed as mean ± standard deviation.

RESULTS

Water content of chicken feed samples

Table 2 presents the water content g/100 of fresh matter (FM) of chicken feed samples. Water content varied from 20.8 ± 16.6 g/100g of FM in the Centre and Littoral to 24.0 ± 5.1 g/100g of FM in the West for local bulk broiler feed samples. A significant difference was observed between water content for local bulk broiler feed from both the Centre and Littoral regions and West region ($p < 0.05$). Local bulk layer feeds showed water content varying from 12.4 ± 0.2 g/100g of fresh matter in the Littoral and West to 16.8 ± 6.6 g/100g of FM in the Centre. A significant difference was observed between water content from both Littoral and West regions and the Centre region ($p < 0.05$) concerning local bulk layer feeds. All the imported feed samples for the three regions presented a water content of 12.4 ± 2.7 g/100 g of FM with no significant difference ($p > 0.05$).

Total aflatoxin, aflatoxin B_1, and Ochratoxin A content in chicken feeds

Table 3 presents total aflatoxin (AFT), aflatoxin B_1 (AFB_1), and Ochratoxin A (OTA) contents in broiler and laying chicken feed from the Centre, Littoral, and West regions. The AFT content of broiler feed varies from 3.9 ± 0.2 (Littoral region) to 19.6 ± 0.3 µg/kg (Centre region). The AFB_1 content of broiler feed varies from 1.6 ± 0.1 (West region) to 19.3 ± 0.2 µg/kg (Central region). The OTA content of broiler feed ranges from 1.1 ± 0.01 to 0.8 ± 0.01µg/kg. In the layer feed, the AFT content varies from 3.5 ± 0.1 to 12.6 ± 0.2 µg/kg, the AFB_1 content from 2.8 ± 0.1 to 11.4 ± 0, 2µg/kg, and OTA content from 0.8 ± 0.01 to 1.1 ± 0.001µg/kg.

The total aflatoxin (AFs) content of broiler and layer feed is higher in the Centre region and low in the Littoral region. Aflatoxin B_1 (AFB_1) content is always higher in the Centre and low in the Littoral for broiler feed, raised in the Centre region and lower in the Littoral region for layer feed. In terms of Ochratoxin A (OTA) content, the highest value is presented by broiler feed from the Centre and Littoral regions, while the Littoral region had the highest value in layer feed (Table 3). Table 3 shows a variation between the values of the levels of different toxins from one region to another. This variation results in some cases in a significant difference ($p < 0.05$). This variation in the contents of AFT, $AFB_{1,}$ and OTA can be explained by the different level of contamination of the different ingredients used in the composition of chicken feed and climatic conditions. However, significant differences were also observed between ochratoxin A contents in sample feeds from each region ($p < 0.05$).

Heavy metal content of chicken feed samples

Heavy metals analyzed were of two groups, including essential (Zn, Cu, Chromium, and Nickel) and toxic metals (Lead, Arsenic, Cd, and Hg). Average contents of each metal of each group in different bulk chicken feeds are presented in Tables 4, 5, and 6. Concerning essential metals, Zn showed the highest average content (1587168.5 ± 49.5 µg/kg), while Nishowed the lowest content (8275.7 ± 21.5 µg/kg) as presented in Table 4. Significant differences were not observed between heavy metal contents in imported bulk broiler feed from each region ($p > 0.05$). Table 5 presents the average contents of non-toxic heavy metals in both bulk local layer and broiler feeds from the Centre, Littoral and West regions. As shown in Table 5, significant differences were observed between heavy metal contents of non-toxic heavy metals in bulk local layer and broiler feeds from the three regions ($p < 0.05$). However, bulk local layer and broiler feeds showed the highest average concentration of Zn while Cr presented the lowest average content. Furthermore, significant differences were observed between Nickel, Zn, Cu, and Cr contents in the bulk feed from the Littoral and West regions ($p < 0.05$). These differences were probably due to the diverse sources of the raw materials of the ingredients used to produce feeds.

Table 6 presents the average contents of toxic heavy metals in bulk layer and broiler feeds from the three regions. Significant differences were observed between the average contents for each metal and from each region ($p < 0.05$). The Pb showed the highest average content in bulk local layer feed samples from each region, while Arsenic (As) obtained the lowest average content. In bulk local broiler feed samples, As showed the highest average contents and Cd had the lowest average contents. This clearly shows that the content levels of bulk feed samples are different in terms of chicken type ($p < 0.05$).

Table 2. Water content (g/100 g) of fresh matter of different bulk samples in the three regions of Cameroon

Sample \ Regions	Centre	Littoral	West
Local broiler feed (g/100 g of FM)	20.8 ± 0.6^{aA}	20.8 ± 0.5^{aA}	24.0 ± 0.1^{bA}
Local layer feed (g/100 g of FM)	16.8 ± 0.6^{B}	12.4 ± 0.2^{B}	12.4 ± 0.2^{bB}
Imported layer feed (g/100 g of FM)	12.4 ± 0.7^{aC}	12.4 ± 0.7^{aC}	12.4 ± 0.7^{ab}

FM: Fresh matter, ^{a,b,c} Significant difference in the same column ($p < 0.05$), $^{A, B, C}$ Significant difference in the same row ($p < 0.05$)

Table 3. Total aflatoxins, Aflatoxin B_1, and Ochratoxin A contents in bulk chicken feed samples collected in some poultry farms from the Centre, the Littoral and the West regions of Cameroon

Mycotoxins	Region	Mycotoxin content (µg/kg)								
		Local broiler feed			Local layer feed			Imported layer feed		
		Mean ± SD	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max
Total Aflatoxin (AFs)	Centre	$19.6^{a}\pm0.3$	17.2	20.7	$12.6^{b}\pm0.2$	11.9	13.3	$8.2^{c}\pm1.4$	6.4	9.6
	Littoral	$3.9^{a}\pm0.2$	2.7	5.2	$3.5^{a}\pm0.1$	3.2	3.8	$8.2^{b}\pm1.4$	6.4	9.6
	West	$7.4^{a}\pm0.1$	6.3	8.4	$4.7^{b}\pm0.1$	2.4	8.1	$8.2^{c}\pm1.4$	6.4	9.6
Aflatoxin B_1 (AFB_1)	Centre	$19.3^{a}\pm0.2$	17.6	21.0	$11.4^{b}\pm0.2$	8.7	14.1	$3.6^{c}\pm0.4$	3.1	4
	Littoral	$3.7^{a}\pm0.1$	3.4	4.1	$2..8^{b}\pm0.1$	1.6	3.9	$3.6^{a}\pm0.4$	3.1	4
	West	$1.6^{a}\pm0.2$	1.4	1.8	$3.3^{b}\pm0.1$	2.9	4.9	$3.6^{b}\pm0.4$	3.1	4
Ochratoxine A (OTA)	Centre	$1.1^{a}\pm0.01$	1.0	1.4	$0.9^{a}\pm0.01$	0.8	1.1	$2.6^{b}\pm0.4$b	1.8	3
	Littoral	$1.1^{a}\pm0.01$	1.0	1.3	$1.1^{a}\pm0.01$	1.0	1.2	$2.6^{b}\pm0.4$	1.8	3
	West	$0.8^{a}\pm0.01$	0.6	0.9	$0.8^{a}\pm0.01$	0.6	0.9	$2.6^{b}\pm0.4$	1.8	3

Significant difference between different letters in the same row ($p < 0.05$)

Table 4. Essential and toxic heavy metals in a bulk imported layer feed (µg/kg)

Heavy metals		Average concentration (µg/kg)
Essentials	Cu	248967.8 ± 78.1
	Cr	7760.8 ± 47.7
	Ni	8275.7 ± 21.5
	Zn	1587168.5 ± 49.5
Toxics	As	1.0 ± 0.2
	Cd	2.5 ± 0.3
	Pb	3229.8 ± 3.0
	Hg	4.9 ± 0.6

Cd: Cadmium, Ni: Nickel, Pb: Lead, Cr: Chromium, As: Arsenic, Hg: Mercury, Zn: Zinc, Cu: Copper

Table 5. Average concentrations of non-toxic heavy metals (µg/kg) in bulk local broiler and layer feeds from the three regions of Cameroon

Non-toxic heavy metals	Centre		Littoral		West	
	Bulk broiler feed	Bulk layer feed	Bulk broiler feed	Bulk layer feed	Bulk broiler feed	Bulk layer feed
Ni	22575 ± 35.4^{aA}	6942.4 ± 0.1^{aA}	22522.3 ± 0.4^{bB}	7349.3 ± 0.4^{bB}	22561 ± 55.2^{cC}	7145.8 ± 287.4^{cC}
Zn	82791.7 ± 0.2^{aA}	51789.4 ± 0.1^{aA}	82834.4 ± 0.1^{bB}	54537.4 ± 0.1^{bB}	82813 ± 30.4^{cC}	4150.8 ± 0.4^{cC}
Cu	17760.2 ± 0.5^{aA}	7963.4 ± 0.1^{aA}	17737.5 ± 0.0^{bB}	8370.8 ± 0.4^{bB}	17749 ± 16.3^{cC}	8167.3 ± 288.1^{cC}
Cr	1867.8 ± 0.4^{aA}	3957.2 ± 0.2^{aA}	1882.2 ± 0.07^{bB}	4150.8 ± 0.4^{bB}	1875.1 ± 10.0^{cC}	4054 ± 137.2^{cC}

^{a,b,c} Significant difference in the same column ($p < 0.05$), $^{A, B, C}$ Significant difference in the same row ($p < 0.05$), Ni: Nickel, Zn: Zinc, Cu: Copper Cr: Chromium

Table 6. Average concentrations of toxic heavy metals (µg/kg) in bulk local broiler and layer feeds from the three regions of Cameroon

Toxic heavy metals	Centre		Littoral		West	
	Bulk broiler feed	Bulk layer feed	Bulk broiler feed	Bulk layer feed	Bulk broiler feed	Bulk layer feed
Pb	10 ± 0.0^{aA}	10 ± 0.0^{aA}	7.5 ± 0.0^{bB}	995.8 ± 0.4^{bB}	8.8 ± 1.8^{cC}	503 ± 209.3^{cC}
As	2818.8 ± 0.4^{aA}	2.2 ± 0.0^{aA}	2819.4 ± 0.1^{bB}	2.0 ± 0.07^{bB}	2819.3 ± 0.4^{cC}	2.1 ± 0.1^{cC}
Cd	2.8 ± 0.07^{aA}	2.4 ± 0.0^{aA}	2.7 ± 0.0^{bB}	3.3 ± 0.0^{bB}	2.7 ± 0.0^{cC}	2.9 ± 0.6^{cC}
Hg	4.6 ± 0.07^{aA}	5.6 ± 0.0^{aA}	4.3 ± 0.0^{bB}	5 ± 0.0^{bB}	4.4 ± 0.1^{cC}	5.3 ± 0.4^{cC}

A significant difference between identical letters in the same column and different letters in the same line ($p < 0.05$), Pb: Lead, As: Arsenic, Cd: Cadmium, Hg: Mercury

DISCUSSION

Total aflatoxins, Aflatoxin B_1, and Ochratoxin A in chicken feed samples

This study was conducted in the Centre, Littoral, and West regions of Cameroon, namely Centre, Littoral and West. Total aflatoxins (AFs), Aflatoxin B_1 (AFB_1), and Ochratoxin A (OTA) contents in chicken feed samples, their average content globally respected the recommended standard. The recommended concentrations of AFs, AFB_1, and OTA in poultry feeds (20 µg/kg, 10 µg/kg, and 5µg/kg, respectively, FAO/WHO, 2017; Mokubedi et al., 2019) were higher than concentrations found for different feed samples in this study. This is probably because these feeds were well stored at the farm. During sample collection, it was observed that feeds are stored in places that are not humid and are mostly made for immediate use (2 to 3 days). However, the results of this study are different from previous studies in Guyana ($27380 \pm 82120 \times 10^{-3}$ µg/kg) by Mokubedi et al. (2019), in Nigeria (127400×10^{-3} µg/kg) by Akinmusire et al. (2018), and Cameroon (30000×10^{-3} and 22000×10^{-3} µg/kg) by Abia et al. (2013a) for AFs. Aflatoxin B_1 (AFB_1) content of broiler and layer feed in the Centre region is higher than in other regions. In addition, this content is also higher than the maximum limit for AFB_1 in chicken feed (10µg/kg) set by the Commission of the European Union and the Food and Drug Administration of the United States of America in 2010 (FAO/WHO, 2017). This AFB_1 content represents 193% of 10µg/kg in broiler feed and 114% in layer feed from the Centre region. The obtained results might probably be the consequence of conditions in which feed samples are produced or stored, which promote this toxin production by molds, such as *Aspergillus* whose presence in feed has already been reported (FAO/WHO, 2017). In fact, in the Center region, it was observed that food took longer on the farm, compared to the other two regions. The concentrations of OTA detected in all chicken feed were below the maximum tolerable limit of 5µg/Kg (Morrison et al., 2017). Previous studies in Nigeria and Cameroon reported the contamination of chicken feed or poultry by OTA at variable concentrations of 1200×10^{-3} and 2100×10^{-3} µg/kg (Abia et al., 2013b) and 5400×10^{-3} µg/kg (Akinmusire et al., 2018). Mycotoxins can be carried over from feed to animal body and be bio-accumulated (Mokubedi et al., 2019). Hence, although values are globally lower than the norm, it is suggested that measures should be taken to minimize mold contamination of poultry feeds.

Heavy metals in feed samples

Analysis of heavy metals was carried out in two groups of essential and toxic metals. The concentrations of Zn in different local bulk feed samples were above the maximum acceptable Zn concentration of 3 mg/kg (3000 µg/kg) established by the World Health Organization (WHO, 2011). Compared to the permissible concentration of 2 mg/kg (2000 µg/kg) for Cu in feed asserted by the WHO (2011), the mean concentrations of Cu in all feeds were above. Similar to Zn, Cu is required for many biological processes, including enzyme functions as well as a positive influence on livestock growth and reproduction. Due to the variation of their bioavailability, supplementation of Zn and Cu is necessary for most livestock species (EC, 2003a; EC, 2003b). A similar result was reported by Okoye et al. (2011) in Nigeria. Nickel average concentrations were higher than those reported by Okoye et al. (2011) in Nigeria, ranging from 2250 to 4875 µg/kg and higher than 70 µg/kg in feeds (WHO, 2011). The imported layer feed showed the highest mean concentration for Cr (7760.8 ± 47.7 µg/kg) than any other feed sample. Chromium concentrations in different feed samples were above the permissible limit set by WHO (2011) of 50 µg/kg in feeds.

Bulk broiler feed samples from the three regions showed an average concentration of Arsenic above the permissible concentration (500 µg/kg, Nachman et al., 2005). The level of Cd in the bulk layer feed from the two poultry farms in the Littoral region and the second poultry farm in the West was above the permissible concentration of 3 µg/kg in feed (WHO, 2011). The Commission Directive 2005/8/EC permits a maximum Hg 0.1 mg/kg (100µg/kg) for complete feedstuffs (EC, 2005). The current study indicated that all bulk feed samples showed Hg average concentrations above this maximum allowed limit. Islam et al. (2007) reported the presence of Hg at the concentration of 57.9 µg/kg and 11.6 µg/kg in different types of poultry feed produced in Bangladesh. The permissible Pb limit set by WHO (2011) is 10 µg/kg. Bulk layer feed from the Littoral and West region was above the permissible limit. These low values of heavy metals, particularly toxic metals, could be bio-accumulated in chicken tissues and eggs during their life and be responsible for health concerns as reported by the CFIA (2017) and Tatfo Keutchatang et al. (2022). Contaminants can be accumulated in chicken tissues and eggs used for human consumption.

CONCLUSION

Feeds used in chicken farming for broilers and egg production were contaminated by mycotoxins (total aflatoxins, aflatoxin $B_{1,}$ and ochratoxin A) and both essential and toxic metals in the study area (Centre, Littoral, and Western regions of Cameroon). The contents of these contaminants were, in a few cases, above the recommended or permissible limits. This situation could lead to the presence of their residues in chicken tissues and eggs responsible for health concerns and the low productivity of the poultry sector in Cameroon.

DECLARATIONS

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Authors' contributions

Fabrice De Paul Tatfo Keutchatang, and Isabelle Sandrine Bouelet Nstama drafted the research protocol, collected data, and drafted the manuscript under the guidance of Gabriel Medoua Nama and Germain KANSCI. Data were analyzed by Borelle Mafogang Alex Dimitri Tchuenchieu Kamgain, Evelyne Nguegwouo, Hippolyte Mouafo Tene, and Fabrice De Paul Tatfo Keutchatang. All activities were supervised by Gabriel Medoua Nama and Germain KANSCI. All the authors edited the manuscript and approved its final content.

Competing interests

The authors declare that they have no conflicts of interest.

Ethical considerations

All ethical issues, including concerns to publish, data falsification, reuse of data already polished, misconduct, plagiarism, and redundancy were taken into consideration and have been verified and checked by the authors

Availability of data and materials

The data of the article will be sent by the corresponding author according to reasonable requests.

REFERENCES

Abia WA, Simo GN, Warth B, Suyolk M, Krska R, Tchana A, and Moundipa PF (2013a). Determination of multiple mycotoxins levels in poultry feeds from Cameroon. Japan Journal of Veterinary Research, 61(Suppl): S33-S39. Available at: https://eprints.lib.hokudai.ac.jp/dspace/bitstream/2115/52353/3/JJVR61-S_PROC.pdf

Abia WA, Warth B, Sulyok M, Krska R, Tchana AN, Njobeh PB, Dutton MF, and Moundipa PF (2013b). Determination of multi-mycotoxin occurrence in cereals, nuts and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS). Food Control, 31(2): 438-453. DOI: https://www.doi.org/10.1016/j.foodcont.2012.10.006

Akinmusire OO, El-Yuguda AD, Musa JA, Oyedele OA, Sulyok M, Somorin YM, Ezekiel CN, and Krska R (2018). Mycotoxins in poultryfeed and feedingredients in Nigeria. Mycotoxin Research, 35(2): 149-155. DOI: https://www.doi.org/10.1007/s12550-018-0337-y

Aral Y, Aydin E, Demir P, Akin AC, Cevger Y, Kuyululu ÇY, and Arikan MS (2013). Consumer preferences and consumption situation of chicken meat in Ankara province, Turkey. Turkish Journal of Veterinary and Animal Sciences, 37(5): 582-587. Available at: https://journals.tubitak.gov.tr/cgi/viewcontent.cgi?article=1980&context=veterinary

Association of official analytical chemists (AOAC) (2000). Official methods of analysis. USA, p. 25.

Becer UK and Filazi A (2010). Aflatoxins, nitrates and nitrites analysis in the commercial cat and dog foods. Fresenius Environmental Bulletin, 19(11): 2523-2527.

Broekaert JAC (2005). Analytical atomic spectrometry flames and Plasmas, 2nd Edition. DOI: https://www.doi.org/10.1002/3527606653

Canadian food inspection agency (CFIA) (2017). RG-8 regulatory guidance: Contaminants in feed (Formerly RG-1, Chapter7) Section 4: Metal contamination. Available at: https://www.inspection.gc.ca/animal-health/livestock-feeds/regulatory-guidance/rg 8/eng/1347383943203/1347384015909

Centre d'expertise en analyse environnementale du Québec (CEAEQ) (2015). Available at: www.ceaeq.gouv.qc.ca/index.asp

Demirezen D and Uruc K (2006). Comparative study of trace elements in certain fish, meat and meat products. Meat Science, 74(2): 255-260. DOI: http://www.doi.org/10.1016/j.meatsci.2006.03.012

European Commission (EC) (2003a). Opinion of the scientific committee on animal nutrition on the use of zinc in feedstuffs. European commission, health and consumer protection directorate, Brussels, Belgium. Available at: https://lavasoft.gosearchresults.com/?q=%3B+EC%29+European+Commission+%282003a%29.&tt=vmn__webcompa__1_0__go__lvs__webcompa__1_0__go__ch_WCYID10420_190423__yrff__yrff&pid=5ac784309091147a162b4431

European commission (EC) (2003b). Opinion of the scientific committee on animal nutrition on the use of copper in feedstuffs. European commission, health and consumer protection directorate. Brussels, Belgium. Available at: https://food.ec.europa.eu/system/files/2020-12/sci-com_scan-old_report_out115.pdf

European commission (EC) (2006). Commission regulation No. 401/2006 means commission regulation (EC) No. 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs as amended by commission regulation (EU). Available at: https://www.legislation.gov.uk/eur/2006/401/contents

Food and agricultural organization/ world health organization (FAO/WHO) (2017). Joint FAO/WHO food standards programme, codex committee on contaminants in foods, WHO, Geneva, Switzerland. Available at: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-735-11%252FREPORT%252FREP17_CFe.pdf

Guetiya Wadoum RE, Zambou NF, Anyangwe FF, Njimou JR, Coman MM, Verdenelli MC, Cecchini C, Silvi S, Orpianesi C, Cresci A et al. (2016). Abusive use of antibiotics in poultry farming in Cameroon and the public health implications. British Poultry Science, 57(4): 483-493. DOI: https://www.doi.org/10.1080/00071668.2016.1180668

Hazrat A, Ezzat K, and Ikram I (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation. Journal of Chemistry, 2019: 673030. DOI: https://www.doi.org/10.1155/2019/6730305

Höffler H (2018). Poultry production in Cameroon: How the import restriction affects the Cameroonian poultry sector. Deutsche Gesellschaftfür Internationale Zusammenarbeit (GIZ) GmbH, sector project agricultural trade and value chains, SNRD-Asia, Bangkok, Thailand, p. 12. Available at: https://www.giz.de/de/downloads/GIZ_SVAAA_Policy-Brief-Cameroon-Chicken_EN.pdf

Islam MS, Kazi MA, Hossain MM, Ahsan MA, and Hossain AM (2007). Propagation of heavy metals in poultry feed production in Bangladesh. Bangladesh Journal of Scientific and Industrial Research, 42(4): 465-474. DOI: https://doi.org/10.3329/BJSIR.V42I4.755

Kaya S (2014). Mycotoxins. In: S. Kaya (Editor), Veterinary toxicology, 3rd Edition. Medisan Publisher., Ankara, Turkey. pp. 393-433.

Mokubedi SM, Phoku JZ, Changwa RN, Gbashi SPB, and Njobeh PB (2019). Analysis of mycotoxins contamination in poultry feeds manufactured in selected provinces of South Africa using UHPLG-MS/MS. Toxins, 11(8): 452. DOI: https://www.doi.org/10.3390/toxins11080452

Morrison DM, Ledoux DR, Chester LFB, and Samuels CAN (2017). A limited survey of aflatoxins in poultry feed and feed ingredients in Guyana. Veterinary Sciences, 4(4): 60. DOI: https://www.doi.org/10.3390/vetsci4040060

Nachman KE, graham JP, Price Lb, and Silbergeld KL (2005). Arsenic: A roadblock to potential animal waste management solutions. Environmental Health Perspectives, 113(9): 1123-1124. DOI: https://www.doi.org/10.1289/ehp.7834

Okoye COB, Ibeto CN, and Ihedioha JN (2011). Assessment of heavy metals in chicken feeds sold in south eastern Nigeria. Advances in Applied Science Research, 2(3): 63-68. Available at: https://www.primescholars.com/articles/assessment-of-heavy-metals-in-chicken-feeds-sold-in-south-eastern-nigeria.pdf

Paryad A, and Mahmoudi M (2008). Effect of different levels of supplemental yeast (Saccharomyces cerevisiae) on performance, blood constituents and carcass characteristics of broiler chicks. African Journal of Agricultural Research, 3: 835-842. Available at: https://academicjournals.org/journal/AJAR/article-stat/AD3847E38476

Scientific committee for animal nutrition (SCAN) (2003). Summary record of the 149th scan plenary meeting. Available at: https://ec.europa.eu/food/system/files/2020-12/sci-com_scan-old_sum_out131.pdf

Tatfo Keutchatang FDP, Sandrine IBN, MedouaNG, and Kansci G (2021). Biosecurity practices and characteristics of poultry farms in three regions of Cameroon. Journal of World Poultry Research, 11(1): 64-72. DOI: https://www.doi.org/10.36380/jwpr.2021.9

Tatfo Keutchatang FDP, Tchuenchieu KA, Nguegwouo E, Tene MH, Sandrine IBN, Kansci G, and Medoua NG (2022**).** Occurrence of total aflatoxins, aflatoxin B1, and ochratoxin A in chicken and eggs in some Cameroon urban areas and population dietary exposure. Journal of Environmental and Public Health, 2022: 5541049. DOI: https://www.doi.org/10.1155/2022/5541049

World health organization (WHO) (2011). Influenza at the human-animal interface. Summary and assessment, 5 December 2011. Available at: https://www.who.int/publications/m/item/influenza-at-the-human-animal-interface-summary-and-assessment-5-dec-2011

World health organization (WHO) (2017). What is one health? Available at: https://www.onehealthcommission.org/en/why_one_health/what_is_one_health/