agriculture

MDPI

Article

Mycotoxicological Assessment of Broiler Compound Feed: A Multi-Year Analysis of Five Mycotoxins in a Romanian Feed Mill

Dragoș Mihai Lăpușneanu, Silvia-Ioana Petrescu *, Cristina-Gabriela Radu-Rusu, Mădălina Matei and Ioan Mircea Pop

Department of Control, Expertise and Services, Faculty of Food and Animal Sciences, "Ion Ionescu de la Brad" Iasi University of Life Sciences, 8 Mihail Sadoveanu Alley, 700489 Iasi, Romania; dragos.lapusneanu@iuls.ro (D.M.L.); cristina.radurusu@iuls.ro (C.-G.R.-R.); madalina.matei@iuls.ro (M.M.); mircea.pop@iuls.ro (I.M.P.)

* Correspondence: silvia.petrescu@iuls.ro

Abstract: Mycotoxins are secondary metabolites of filamentous fungi that cause massive agricultural losses worldwide and constitute a significant health problem for humans and animals. The aim of this five-year study was to investigate the contamination of compound feed for broiler chickens at all stages (starter, grower and finisher) from a feed mill in Romania with mycotoxins such as total aflatoxins (AFT), deoxynivalenol (DON), fumonisins (FUMs), ochratoxin A (OTA) and zearalenone (ZEN). AFT was detected in 49.3–72.2% of the samples with concentrations ranging from 0.01 to 5.2 µg/kg. DON was detected in 77.6–98.9% of the samples, with maximum concentrations ranging from 330 to 1740 µg/kg. FUM contamination ranged from 42.7% to 87.2%, with maximum levels between 460 and 1400 µg/kg. OTA was present in 44.2–87.9% of the samples, with maximum concentrations reaching 21.4 µg/kg. ZEN was consistently elevated at all feeding stages, being detected in 86.5–97.4% of the samples, with maximum levels of 89.4 µg/kg. Mycotoxin co-occurrence was common in the samples, with the most common combination of four mycotoxins occurring in 38.51% of the samples. Samples were collected from storage bunkers, homogenised and analysed in certified laboratories, with sampling procedures varying according to batch size to ensure representativeness. Investigation of the transfer of mycotoxins into animal products and the combined effects of mycotoxins on animal health, including potential synergistic or antagonistic interactions, is particularly relevant. This study emphasises the essential role of comprehensive and continuous monitoring of mycotoxins in protecting animal health and food safety.

Keywords: mycotoxins; compound feed; broiler; co-occurrence; food safety

Academic Editor: Hai Lin

Received: 22 November 2024
Revised: 31 December 2024
Accepted: 31 December 2024
Published: 2 January 2025

Citation: Lăpușneanu, D.M.; Petrescu, S.-I.; Radu-Rusu, C.-G.; Matei, M.; Pop, I.M. Mycotoxicological Assessment of Broiler Compound Feed: A Multi-Year Analysis of Five Mycotoxins in a Romanian Feed Mill. *Agriculture* **2025**, *15*, 84. https://doi.org/10.3390/agriculture15010084

1. Introduction

Food and feed safety is a major concern for both animal and human health due to the frequent contamination of food and feed with various contaminants [1,2].

The word "mycotoxin" is derived from the Greek words "myco" and "toxin", meaning "mould" and "poison" produced by a living organism [3]. Mycotoxins are a group of toxic chemical compounds produced as secondary metabolites by certain mould species, primarily within the genera *Aspergillus*, *Fusarium* and *Penicillium* [4–6].

Based on the literature data, the overall prevalence of mycotoxins in food crops varies widely depending on many factors such as the mycotoxin in question, the analytical methods used and the reporting of results, but the prevalence for detected mycotoxins is reported to be up to 60–80% [7], and this is considered an unavoidable and unpredictable problem that poses a challenge to food safety [8].

Contaminated feed and food products pose high risks to animal health and human metabolic conditions, which can range from acute symptoms of severe disease to long-term effects [9–11]. Because of incidents of mycotoxin poisoning [12,13], most countries or regions have regulatory levels for the presence of mycotoxins in certain food staples or feeds; therefore, testing for those specific regulated mycotoxins is required [14], using specific and selective analytical techniques adapted to verify food safety and protect public health [3]. The maximum levels or guideline levels for mycotoxins in products intended for animal feed in the European Union are highlighted in Table 1, as they have been found in the legislative support mentioned [15,16].

Table 1. European Union mycotoxin limits or guidance levels in animal feed.

Mycotoxin	Products Intended for Animal Feed	Maximum Content/Guidance Value Relative to a Feed with a Moisture Content of 12% (µg/kg)	Legislative Support
Aflatoxin B1	Feed materials	20	Reg. (EU) No 574/2011 [15]
	Compound feed for young poultry	5	
	Compound feed for poultry	20	
Deoxynivalenol	Cereals and cereal products	8000	Reg. (EU) 576/2006 [16]
	Maize by-products	12,000	
	Compound feed for poultry	NR	
Fumonisin B1 + B2	Maize and maize products	60,000	
	Compound feed for poultry	20,000	
Ochratoxin A	Cereals and cereal products	2500	
	Compound feed for poultry	100	
Zearalenone	Cereals and cereal products	2000	
	Maize by-products	3000	
	Compound feed for poultry	NR	

NR = no/without regulation.

Aflatoxins (AFTs) are a class of carcinogenic mycotoxins produced by Aspergillus species, especially *Aspergillus flavus* and *A. parasiticus* [17–19]. When grains such as maize are grown in an environment with high ambient temperatures (day > 32 °C; night > 24 °C), the grains become more susceptible to aflatoxin formation. Maize grains can contain up to 400,000 µg/kg of aflatoxin, so sampling is very important when analysing contamination levels [5]. All primary transformations of aflatoxin B_1 involve conversion to hydroxyl metabolites, the most important resulting toxin in terms of toxicity being aflatoxin M_1. Aflatoxin B_1 is immunosuppressive in animals, with particularly strong effects on cell-mediated immunity. According to expert studies, aflatoxin B_1 is genotoxic, inducing genetic mutations and chromosomal changes [19].

Based on what we know so far, the presence of AFTs in feed leads to suppression of the immune response in birds, onset of oxidative stress and disruption of liver enzyme activity [20]. Furthermore, a recent study highlights the effects of long-term exposure to AFB_1, which may lead to decreased bone density in broiler chickens not only as a result of impaired vitamin D or calcium and phosphorus absorption, but the mycotoxin itself at levels of 230 µg/kg causes decreased bone mass in poultry [21].

Deoxynivalenol (DON), also known as vomitoxin, is produced by *Fusarium geaminearum* and, in certain geographical areas, by *F. culmorum* [22]. The main crops affected are maize and small grains such as wheat, oats and barley. In maize, "stem and ear rot"

caused by *F. graminearum* may appear as purple or pink kernels with visible pink mould growths on affected areas of the cob. Storage under optimum conditions (<14% humidity) will minimise further toxin production by pathogenic fungi [5]. Contamination of feed with DON even in low concentrations, below 1900 µg/kg, can lead to severe intestinal pathologies according to current studies, affecting not only the morphostructural activity of the intestinal villi of broilers but also possibly leading to decreased response of digestive enzymes [23].

Fumonisins (FUMs) include a group of relatively recently discovered mycotoxins (mainly fumonisins B_1, B_2 and B_3), primarily produced by *F. verticillioides* and *F. proliferatum*, with maize being the main commodity affected [24]. Grains damaged by insects, birds or cracked kernels will often contain the highest levels of toxin and cause serious disease in animals [25]. Worldwide reports have documented ppm levels of fumonisin B_1 contamination. Human exposure occurs at levels ranging from micrograms to milligrams per day and is highest in regions where maize products are a staple. Based on toxicological evidence, the IARC (International Agency for Research on Cancer) has classified fumonisin B_1 as possibly carcinogenic to humans (group 2B) [26].

Ochratoxin A (OTA) is a naturally occurring fluorescent compound, and its detection during analysis typically relies on this property [5]. Following aflatoxins, ochratoxin A represents the most significant mycotoxin in terms of its impact. OTA is produced by members of the genera *Aspergillus* (*A. ochraceus, A. carbonarius*) and *Penicillium* (*P. verrucosum*). It has been observed that contamination with OTA is a global phenomenon, as evidenced by studies [27,28]. The initial fungal growth in cereals can result in sufficient moisture through metabolism to allow further growth and mycotoxin formation. Consequently, the toxin may still be present in cereal products, thereby exposing human and animal populations to contamination [5].

The IARC has classified ochratoxin A as Group 2B, possibly carcinogenic to humans, based on certain indicators of carcinogenicity established in experimental animals [29].

Zearalenone (ZEN) is a mycotoxin produced by several *Fusarium* species, in particular *F. graminearum*, *F. culmorum*, *F. cerealis*, *F. equiseti*, *F. verticillioides* and *F. incarnatum* [30], which can cause several diseases in animals [31]. It is commonly found in maize but can also be found in other crops such as wheat, barley, sorghum and rye. In general, *Fusarium* specifically thrives and contaminates crops under wet and cold weather conditions. Although ZEN is primarily a contaminant of field crops, development of the toxin can also occur under inadequate storage conditions [32]. In the 2000s, the European Union's food safety policy was reformulated, in accordance with the approach of an integrative concept "from farm to fork", thus guaranteeing a high level of safety for food products in all stages of the production chain [33]; even feed mills, like food units, must have auto-control programs for contaminants [34,35].

Raw materials, such as cereals, oilseeds, legumes and, in particular, compound feed as complex matrices, are susceptible to contamination with bacterial or fungal mycotoxins [36]. For example, a study conducted in Poland shows that compound feeds for broilers are characterised by higher contamination with mycotoxins mainly belonging to the *trichothecenes* group; grower and finisher feeds are characterised by higher numbers of bacteria and fungi compared to starter feeds [37].

Considering the inclusion of compound feed production in the food chain, the current research highlights the presence of five mycotoxins such as AFT, DON, FUM, OTA and ZEN in samples taken over 5 years from a feed mill in the north of Romania. The results obtained were compared with those reported by other national and international researchers, and also the presence of more than one mycotoxin in the compound feed taken into study was identified and discussed. Although environmental factors (such as drought or heavy

rainfall) and climate change have affected the geographical area indicated in this study, the mycotoxin values found in the samples did not exceed the legislative limits proposed by the European Commission. In addition to identifying potential new mycotoxins, future studies will examine how they affect animal health and how they co-occur in animal feed.

2. Materials and Methods

2.1. Feed Samples

Compound feeds are especially susceptible to contamination with multiple mycotoxins because they are a blend of multiple raw materials [10,38].

The broiler compound feed samples for starter, grower and finisher stages came from a Romanian feed mill that produces 85,000 t/year, which is representative of the country's feed production. In 2019, 284 samples of compound feed were analysed (92 starter, 79 grower and 113 finisher), in 2020, 241 samples were analysed (91 starter, 87 grower and 143 finisher), in 2021, 306 samples were analysed (98 starter, 82 grower and 126 finisher), in 2022, 333 samples were analysed (102 starter, 97 grower and 134 finisher) and in 2023, 350 samples were analysed (95 starter, 103 grower and 152 finisher). Samples of combined feeds were taken from the feed mill's storage bunkers and sent to the in-plant laboratory for analysis.

For the analysis of the compound feed, 4, 7, 11 and 14 incremental samples were manually sampled with a trowel from batches of 24, 48, 72 and 96 tonnes. The lot size dictated how many elementary samples were sampled overall, which were then separated and homogenised using a centrifugal mechanical divider to create the laboratory sample. The incremental sample size was a minimum of 3 kg, and the aggregate sample was made by reducing the incremental sample to a minimum of 0.5 kg. The feed samples were analysed as such. The results obtained were interpreted on a dry matter basis of 88% (12% moisture) in order to be compared with the maximum permissible limits established in the European Union legislation, presented in Table 2.

Table 2. Detection and quantification limits for five mycotoxins.

Mycotoxins	Limits of Detection (LoD) (µg/kg)	Limits of Quantification (LoQ) (µg/kg)
AFT	0.5	0.5
DON	50	50
FUM	50	50
OTA	0.5	0.5
ZEN	10	20

2.2. Equipment Used for Detection

The mycotoxin determination kits contained the following: microtiter plate spectrophotometer (450 nm); graduated cylinder (glass), 100 mL and 250 mL; glassware for preparing sample extract: filter funnel and 50 mL flask; 20 µL, 200 µL and 1000 µL micropipettes; 50 µL, 100 µL and 1000 µL micropipettes; filter paper: Whatman No. 1; scale (measurement range at least up to 50 g and precision of $\pm$0.01 g); centrifuge (at least 3500$\times$ *g*) + centrifugal vials with cap (50 mL centrifuge tubes); vortex mixer 8-channel pipette for 50, 100 and 300 µL; grinder (mill); shaker; Ultra-Turrax.

2.3. Integrated Management System and Personnel Training

In the compound feed mill taken into study, the prevention of nonconformities in all phases under the control of the organisation is achieved by maintaining and continuously

improving the effectiveness of an integrated management system in accordance with the requirements of the referenced standards: SR EN ISO 9001:2015, SR EN ISO 22000:2019 and SR ISO 45001:2018 [39–41]. In concordance with the above listed standards, the establishment is obliged to develop and comply with specific procedures on food quality and safety. In this regard, all team members who used the RIDASCREEN-FAST kit were trained by means of documented procedures (procedure on sampling techniques for laboratory examinations; procedure on quantitative determination of mycotoxins). The training included both theoretical aspects (technical principles of the kit and mycotoxins to be analysed) and practical aspects (correct use of the kit, handling of laboratory equipment, compliance with safety procedures). The training was an ongoing process, which included recap sessions and periodic staff performance evaluations, with the aim of ensuring that staff remained up to date with protocol updates.

2.4. Mycotoxins Analysis

The quantitative determination of mycotoxins was carried out according to the analytical method described in the RIDASCREEN®FAST enzyme immunoassay technical manuals provided by R-Biopharm AG, Darmstadt, Germany. The contamination levels of AFT are the sum of AFB_1, AFB_2, AFG_1 and AFG_2 and of DON and FUM are the sum of FB_1 and FB_2, and OTA and ZEN in the samples were measured using individual RIDASCREEN®FAST laboratory kits.

The extraction methods for mycotoxin determination are different depending on the specific toxin analysed. Total aflatoxins and fumonisins are extracted using 70% methanol, followed by mixing 5.0 g of ground sample with 25.0 mL of methanol. The extract is mixed thoroughly for three minutes and then filtered through filter paper. The resulting filtrate is then diluted with distilled water, containing 1.3 mL of fumonisin and 1 mL of aflatoxin. However, the extraction solvents are different for deoxynivalenol, ochratoxin A and zearalenone. DON is extracted by shaking 5.0 g of sample with 100 mL of distilled water, whereas ochratoxin A requires 50.0 mL of diluted ECO extractor. ZEN also uses 70% methanol, in the same way as AFT, but with a different dilution ratio.

In the case of incubation and washing, the test for AFT requires a 10 min incubation with the enzyme–antibody mixture at room temperature (20–25 °C), whereas DON, FUM and ZEN normally require a 5 min incubation. OTA requires a more complex extraction, with a 5 min mixing step followed by centrifugation, which differentiates it from other mycotoxins in terms of sample preparation.

Washing the wells is performed with 250 µL of buffer solution and by repeating the process twice, and it is similar for all mycotoxins determined. After washing, each well is treated with 100 µL of substrate/chromogen and incubated for varying periods: 3 min for DON, FUM and OTA and 5 min for AFT and ZEN, all at room temperature in the dark. After this incubation, 100 µL of stop solution is added to each well, and the absorbance is measured at 450 nm. For most mycotoxins, absorbance is normally read 10 min after the addition of the stop solution. In the case of OTA, the reading time is extended slightly, again requiring up to 15 min.

2.5. Statistical Analysis

The data obtained from the analyses were statistically processed and interpreted. The minimum and maximum values were determined, and the position and variance estimators, arithmetic mean ($\overline{x}$) and standard deviation (s) were calculated for the samples with positive results. Means and standard deviations were calculated using Microsoft Excel 2016 [42]. The statistical analysis of variance (ANOVA) was conducted using the GraphPad

Prism (9.3.0) program to compare the levels of each mycotoxin across different feed types and the annual averages.

3. Results

The concentrations of AFT, DON, FUM, OTA and ZEN of the starter compound feed are presented in Table 3.

Table 3. Results of mycotoxicological assessment of starter compound feed.

Mycotoxin	Year	No. of Samples	Positive Samples (µg/kg)					
			%	1st Quartile	$\bar{x}$	s	3rd Quartile	Maximum
AFT	2019	92	70.6	0.9	1.6	0.8	2	4.6
	2020	91	57.1	0.3	0.7	0.8	0.95	4.8
	2021	98	63.4	0.4	1.2	0.7	1	4.2
	2022	102	72.2	0.7	0.8	0.9	1.5	4.5
	2023	95	58.5	0.3	0.7	0.6	0.7	3.8
DON	2019	92	89.1	74	99.2	67.6	100	470
	2020	91	98.9	60	155.1	200.1	140	1120
	2021	98	92.3	57	136.3	85.7	230	670
	2022	102	97.4	50	98.4	128.2	120	910
	2023	95	79.6	47	87.2	78.1	100	730
FUM	2019	92	77.1	30	172.3	194.8	222	1230
	2020	91	51.6	10	65.3	97.5	65	460
	2021	98	65.2	50	82.4	113.8	270	820
	2022	102	87.2	65	142.7	189.6	300	1170
	2023	95	55.8	20	75.6	96.2	120	510
OTA	2019	92	82.6	0.62	1	0.7	1.3	3.59
	2020	91	61.5	0.1	0.5	1	0.5	7.4
	2021	98	72.3	0.1	1.2	0.5	1.7	8.2
	2022	102	85.3	0.5	1	0.8	1.4	6.8
	2023	95	57.8	0.2	0.5	1.2	0.9	4.7
ZEN	2019	92	94.5	11.2	19.7	10.4	25	49
	2020	91	90.1	5.1	11.9	11.2	12.6	57.4
	2021	98	95.8	8.3	20.8	13.7	32	45.2
	2022	102	86.5	12	13.9	12.4	27	67.4
	2023	95	97.4	6.5	21.4	10.2	15	52

$\bar{x}$—mean; s—standard deviation.

The most evident contamination in the case of AFT, according to the results obtained, occurred in the year 2022, the year in which most feed samples were tested, but the most significant contamination related to the number of samples tested and with a high maxima of 4.6 or even 4.8 was in the years 2019 and 2020, and also in 2020 we observed a massive contamination of samples with the DON mycotoxin, reaching a maximum of 1120 µg/kg. With the same approach of a ratio of positive samples to the number of samples studied, 2019 is the most significant year for FUM and OTA contamination of broiler starter feed.

Table 4 contains the results obtained for the grower feed with regard to the level of contamination with all mycotoxins investigated.

Table 4. Results of mycotoxicological assessment of grower compound feed.

Mycotoxin	Year	No. of Samples	Positive Samples (µg/kg)					
			%	1st Quartile	$\bar{x}$	s	3rd Quartile	Maximum
AFT	2019	79	68.3	0.7	1.4	0.7	2	2.7
	2020	87	49.4	0.5	0.7	0.5	1.4	2.4
	2021	82	59.8	0.8	1.7	0.8	2.2	2.5
	2022	97	75.3	1	1.9	0.9	3.1	3.9
	2023	103	52.7	0.5	0.8	0.7	2.7	4.2
DON	2019	79	87.3	80	95.4	56.7	100	330
	2020	87	96.5	60	178.3	250.4	152.5	1290
	2021	82	77.6	100	98.5	327.2	370	1480
	2022	97	86.8	120	146.8	94.7	230	580
	2023	103	94.7	90	85.8	112.9	460	740
FUM	2019	79	69.6	110	156.5	176.2	440	912
	2020	87	43.6	65	56.3	72	70.5	300
	2021	82	51.3	90	87.6	162.7	300	500
	2022	97	86.8	60	122.8	134.6	290	972
	2023	103	42.7	50	54.2	82	160	420
OTA	2019	79	79.7	0.65	1.2	1.1	1.63	8.3
	2020	87	52.8	0.55	0.7	1.1	0.6	5.6
	2021	82	87.9	0.9	0.5	1.7	2.7	9.2
	2022	97	44.2	1.4	1.4	2.2	4.6	7.4
	2023	103	62.4	3.8	0.9	1.1	10.2	17.2
ZEN	2019	79	93.6	10.5	18.3	10.1	30	39.2
	2020	87	90.8	10.2	11.6	8.6	15	45
	2021	82	92.3	15.7	23.8	11.3	54.9	72.9
	2022	97	96.2	11.3	17.8	7.3	29.6	48
	2023	103	91.4	12.8	19.4	9.6	27.2	54.4

$\bar{x}$—mean; s—standard deviation.

Considering the results and applying the same rule of reporting the percentage of positive samples to the number of samples studied for the grower feed, we observe that the levels of AFT, DON and ZEN were the highest in the years 2019 and 2020. FUM and OTA recorded higher contamination levels in the years 2021 and 2022.

The results of the mycotoxicological assessment of the finisher compound feed are listed in Table 5. Analysing the results and applying the same rule of a ratio of the percentage of positive samples to the total number of samples studied for the finisher feed, we note a significant contamination with all mycotoxins in the years 2019 and 2020, with contamination reaching even maximum values of 1510 µg/kg for DON in 2020 or 1080 µg/kg for FUM in 2019.

The five-year investigation of mycotoxin contamination in broiler compound feed has revealed a complex pattern of co-occurrence between several mycotoxins, as can be seen in Figure 1. The most frequent co-occurrence involved four mycotoxins detected in 38.51% of the samples. The data highlight the risk of multiple mycotoxin contamination of broiler feed, with a wide range of co-occurrence combinations observed.

Table 5. Results of mycotoxicological assessment of finisher compound feed.

Mycotoxin	Year	No. of Samples	Positive Samples (µg/kg)					
			%	1st Quartile	$\bar{x}$	s	3rd Quartile	Maximum
AFT	2019	113	64.9	0.9	1.5	0.7	2	4.6
	2020	143	51.04	0.7	0.8	0.7	1.2	3.9
	2021	126	69.2	1.3	1.3	0.8	3.7	4.8
	2022	134	49.3	1.8	0.7	0.6	4.2	5.2
	2023	152	53.9	1	1.9	0.7	2.2	3.7
DON	2019	113	91.07	70	90	54.1	90	321
	2020	143	98.6	60	161.7	219.1	160	1510
	2021	126	89.7	90	96	62.4	240	581
	2022	134	93.02	130	176.3	232.3	540	1740
	2023	152	95.4	80	128.5	119.8	470	842
FUM	2019	113	71.4	70	175.2	215.2	222	1080
	2020	143	67.1	60	95	156.5	100	900
	2021	126	61.8	90	150.4	162.3	320	870
	2022	134	78.2	130	184.7	226.3	280	1400
	2023	152	64.9	90	98	165.7	130	960
OTA	2019	113	82.1	0.5	1.4	2.2	1.6	18.5
	2020	143	65.03	0.5	0.8	1.2	0.9	8.5
	2021	126	72.2	1.5	1.2	2	4.2	9.4
	2022	134	63.5	0.8	0.9	1.1	2.8	13.7
	2023	152	84.2	1.3	1.9	2.4	3.7	21.4
ZEN	2019	113	91.9	11.6	19.3	14.3	25	78.7
	2020	143	95.8	10.3	13.4	10.2	17.4	51
	2021	126	89.5	12.4	17.6	15.6	19.2	87.9
	2022	134	93.2	19.6	23.8	13.4	35.7	89.4
	2023	152	94.7	11	14.2	11.4	18.9	62

$\bar{x}$—mean; s—standard deviation.

These compound feeds contain varying proportions of raw materials to provide different nutrient levels, with cereal grains always being the primary component. ANOVA results showed no significant differences ($p < 0.05$) in the levels of each mycotoxin across feed types and annual averages. The high proportion of cereals in all these compound feeds may account for the lack of differences. However, it is anticipated that starter compound feed would have less contamination than finisher compound feed, likely due to the lower amount of maize used in the formulation for young poultry, as maize is the main contributor to mycotoxin contamination.

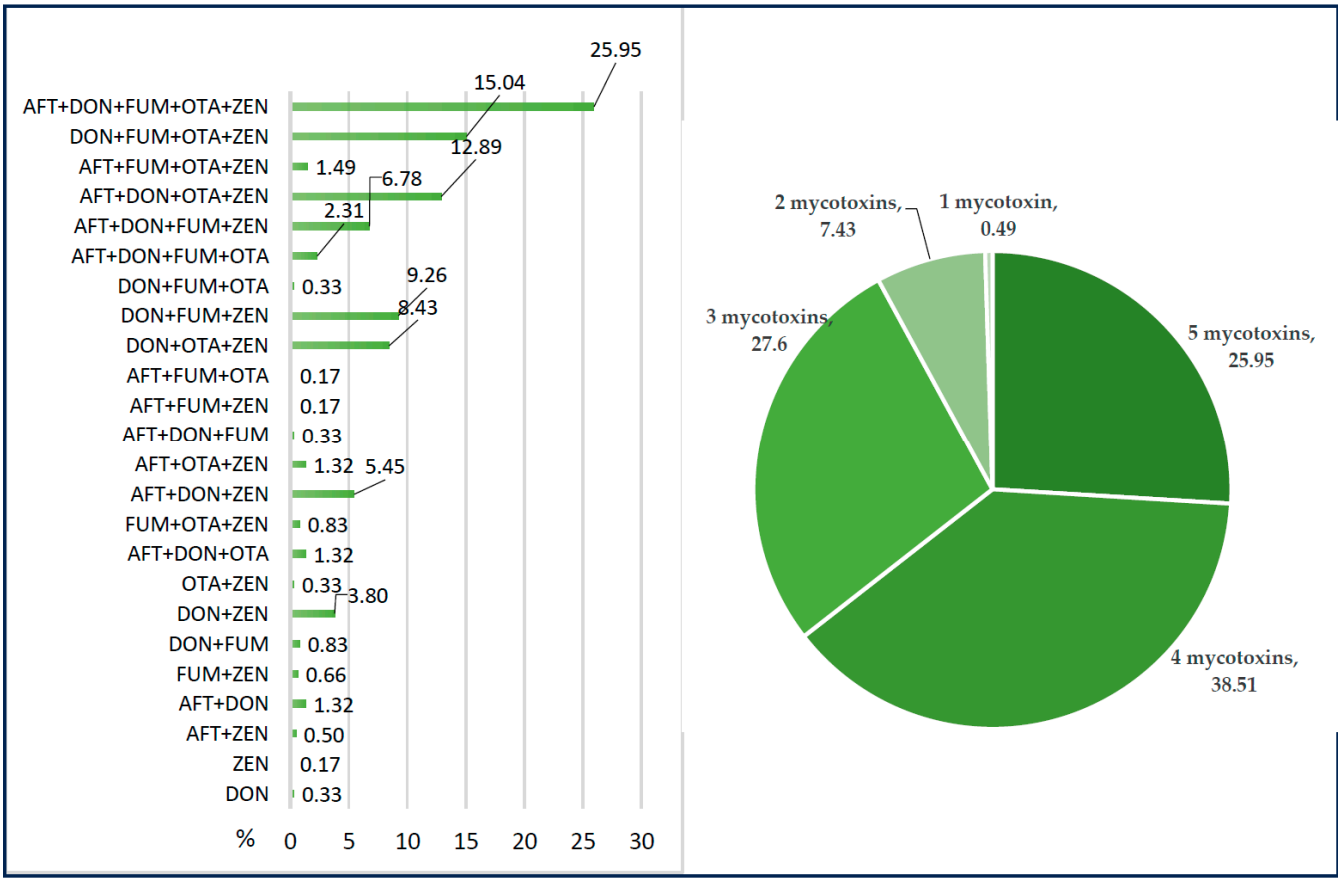

Figure 1. Co-occurrence of mycotoxins in broiler feed over the five-year study period.

4. Discussion

Broiler compound feeds are balanced feed formulas containing a variety of ingredients to ensure complete and healthy nutrition. Main feedstuffs include: maize and wheat flour—used to provide carbohydrates, protein and fibre; soy and sunflower meal—vegetable protein concentrates; vegetable oils (e.g., sunflower oil)—sources of fats, which are essential for energy and overall health of the chickens; salt—added to balance sodium levels; minerals (calcium, phosphorus, magnesium, etc.)—essential for bone and nervous system development; vitamins (A, D3, E, B)—help support the immune system and prevent deficiencies; plant fibre (e.g., wheat bran)—helps the digestive system function properly. The exact composition of compound feed varies depending on the age of the chick, stage of growth and production goals. Typically, broiler feeds are formulated to ensure fast and efficient growth while maintaining poultry health.

Mycotoxins in feed are a significant problem for animal and human safety, and recent studies have shown that food and feed contamination is the rule rather than the exception, impacting all segments of society, from farmers and feed producers to the general public [3,43–45]. Food and feed can be vectors for harmful bacteria, viruses or chemicals that are responsible for a wide range of human and animal health diseases [46,47]. Mycotoxins are secondary metabolites of filamentous fungi that cause massive losses to agriculture worldwide. AFT, OTA, DON, FUM, ZEN and trichothecenes are currently the most commonly tested in the food and feed safety industry [48].

In addition, the range of fungal species that produce these toxins is wide, including *Fusarium*, *Aspergillus* and *Penicillium* species. A two-year study assessed yeast and mould contamination of raw materials and compound feeds; in starter compound feeds, the genus *Aspergillus* was predominant in 2019 (46.6%), while in 2020, species of the genera *Penicillium*

and *Cladosporium* were identified in the majority of samples (50%); for combined feeds for growing and finishing, the genus *Aspergillus* was predominantly identified in 2019 (60% and 72.2% of samples, respectively) and 2020 (61.5% and 46.6%, respectively) [49]. For the 60 most common mycotoxins found in feed, 48% were shown to be produced by the genus *Fusarium*, 13% by the genus *Aspergillus*, 8% by the genus *Penicillium* and 12% by the genus *Alternaria* [50].

In a study conducted by Shar et al. [51], it was found that the natural occurrence of toxins belonging to the genus *Fusarium* in compound feed was similar to that in the raw materials used in their production. The incidence of mycotoxins in feed followed the following order: ZEN > FUM > DON.

In the current research, the highest prevalence in feed was the mycotoxin ZEN (93%), followed by DON (91%) and then OTA, which was identified in 70% of the total samples studied. The incidence of mycotoxins in starter, grower and finisher compound feeds followed the following order: ZEN > DON > OTA.

Although there are hundreds of mycotoxins, regulatory limits or recommendations for maximum tolerated levels in food and feed have been established for only a small number of them [52]. The recognition that mycotoxins affect the health and productivity of poultry and pigs has led to the introduction of regulations setting maximum permissible limits for aflatoxins and guideline recommendations (recommended tolerance levels) for ochratoxins and a small number of fusariotoxins. The limits vary not only according to mycotoxin type, animal species, intended use, feed materials and feedstuffs but also according to regulatory organisation or country; the European Union has established guidelines for feed materials and feedstuffs, with differences depending on the age of the animal and the stage of production [53].

The main aflatoxins consist of aflatoxins B_1, B_2, G_1 and G_2 and can be produced by select isolates of *A. flavus* or *A. parasiticus* [25,54,55]. The Rapid Alert System for Food and Feed (RASFF) reported 5045 and 439 notifications of mycotoxin contamination of food and feed exported to European Union countries worldwide during 2010–2019, respectively, and approximately 89% of mycotoxin contamination notifications of food and 98.6% of feed contamination notifications were attributed to AFT contamination [56]. Averaging the results obtained in all five years taken in study for the feed tested from the feed mill, the maximum AFT content of the combined starter, grower and finisher feed samples was 2.4 μg/kg and reached 5.2 μg/kg for the finisher feed for the year 2022.

The European Commission has established a maximum level for aflatoxin B1 of 20 μg/kg for feed materials, 10 μg/kg for complementary and complete feedingstuffs, 5 μg/kg for compound feedingstuffs intended for chickens and young birds and 20 μg/kg for compound feedingstuffs for poultry (except young animals) [15]. For many years, these toxins were not considered a problem in European agricultural production until early 2013, when aflatoxins in maize for animal feed from the Balkan area caused serious problems in Europe [57,58]. In the present study, we did not identify AFT in concentrations above a maximum permitted level set by the European Commission.

In a study by Greco et al. [59], 44 out of 49 samples of compound feed for broiler chickens were contaminated with aflatoxins, with an average level of 2.685 μg/kg. Decastelli [60] analysed 616 feed samples, and AFT was found in 44 (7%) of the samples. Martins et al. [61] analysed poultry feed and found that 10% and 22%, respectively, were contaminated with AFB_1 at concentrations of 1–21 μg/kg. Šegvić Klarić et al. [62] determined AFT in feed in the range 4.2–10.3 μg/kg (mean 6.9 μg/kg) in 4 (31%) out of 13 samples. In our study, the mean levels of AFT contamination in the combined feed samples were lower than the results reported by previous studies.

Current research highlights a new way of minimising the presence of mycotoxins in complete animal feed; namely, in the case of AFT, various combinations of fruit pomace are used to minimise the number of mycotoxins, the most common being forest fruits [63].

DON is mainly produced by *F. graminearum* and, in some geographical areas, by *F. culmorum* [59]. The average results for DON in the current study were between 321 and 1740 µg/kg in all five years of the combined feed samples; these results were compared with the European limit values and did not exceed them, but in a recent study published by the EFSA regarding DON contamination in poultry feed, since 2017 the EFSA has recommended limits of 600 µg/kg for broilers and turkeys, with effects on gut health and growth suppression in broilers at concentrations of less than 1900 µg/kg DON in their feed [64]. While the current research results show that DON levels in broiler feed samples were below the European regulatory limits for compound feed, EFSA's recommendations underline the need for more stringent limits designed for the biological sensitivity of broilers. A study conducted by Greco et al. [59] shows that 44 out of 49 samples (90%) were contaminated with DON (median 222 µg/kg). In a study on feed, Cegielska-Radziejewska et al. [37] examined poultry feed samples and detected DON contamination in all samples in the range of 3.1–99.4 µg/kg (median 33.6 µg/kg). In another study, DON was found in 56% of poultry feeds, and the median concentration was 303 µg/kg [65]. Driehuis et al. [66] analysed 72 feed samples, and DON was found in 54% of the samples with a maximum concentration of 2408 µg/kg (mean 433 µg/kg). In our study, the average levels of DON contamination were higher than those found by Cegielska-Radziejewska et al. [37] and lower than the results obtained by Labuda et al. and Driehuis et al. [65,66].

FUMs are a group of mycotoxins (mainly FB_1, FB_2 and FB_3) produced mainly by *Fusarium verticillioides* and *F. proliferatum*, and maize is the main commodity affected by this group of toxins [25]. In the present study, the maximum FUM contents in the compound feed samples for starters, growers and finishers were between 300 µg/kg and 1400 µg/kg, with the highest values identified in 2022 in the compound feed for finishers. The European Commission established a guideline value of 60,000 µg/kg for the sum of FB_1 and FB_2 in maize feed materials and 20,000 µg/kg for complementary and complete feed for poultry [15]. In the present study, we did not detect FUMs at concentrations higher than the guideline values established by the European Commission.

A study conducted by Greco et al. [59] shows that fumonisins were detected in all samples analysed in a range of 222–6000 µg/kg, and Martins et al. [61] found that 1% of 337 poultry feed samples were contaminated with FB_1 in a range of 24–34 µg/kg. In another study, Almeida et al. [67] analysed 127 compound feed samples, and FUM was detected in 9% of the samples at a maximum content of 390 µg/kg (median 164 µg/kg). Another study found that FUM in compound feed for broilers had a mean concentration of 304 µg/kg (maximum 1160 µg/kg) in 49 out of 50 samples [66]. Zachariasova et al. [68] analysed 70 samples of poultry and pig feed, and FUM was detected at a maximum content of 10 µg/kg. In their study, Šegvić Klarić et al. [62] found that 7 out of 13 feed samples were contaminated with FUM, and their average was 2300 µg/kg, and the maximum content was 5000 µg/kg. In the present study, the average concentration of FUM in all the compound feed samples was lower than most of the results reported by previous studies.

OTA is a mycotoxin mainly produced by *P. verrucosum* and *A. ochraceus* [25]. In the current research, the maximum OTA content of combined starter, grower and finisher feed samples was determined, and the determined values ranged from 3.5 µg/kg to 21.4 µg/kg for all years included in this study. The European Commission has set a guideline limit value of 250 µg/kg for OTA in feed materials represented by cereals and cereal products and 100 µg/kg for complementary and compound feed for poultry [15]. In a study, Jaimez et al. [69] evaluated the occurrence of OTA in 22 samples of poultry feed; 43% of poultry

feeds were contaminated with OTA at a mean content of 0.50 µg/kg. In a study about concentration of mycotoxins in broiler feed, Martins et al. [61] analysed 100 samples of poultry and pigs feed samples, and OTA was found in one sample at concentration of 4 µg/kg. In our study, the mean contamination levels of OTA in compound feed samples were higher than the results reported by Jaimez et al. [69] and lower than the results reported by Martins et al. [61].

ZEN is one of the most common mycotoxins, being mainly produced by *F. graminearum* and *F. culmorum* [25]. In this study, the maximum ZEN content found in the combined starter, grower and finisher feed samples was averaged over all years and ranged from 39.2 µg/kg to 89.4 µg/kg. The European Commission has set a guideline level of 2000 µg/kg for ZEN in feed materials from cereals and cereal products and 3000 µg/kg for maize by-products. The legal limits for ZEN for broilers are not found in the European Commission regulations; therefore, most research is based on limits based on clinical observations from studies conducted over time on poultry; therefore, the limits for ZEN in feed are very varied, ranging from 4 to 11,192 µg/kg [70]. In the current study, we did not detect ZEN at concentrations higher than the limit values observed in the clinical studies. Although the mycotoxins levels found in this study were below the regulatory limits set for broiler feed, these limitations are not always biologically safe. Chronic exposure to mycotoxins at subregulatory levels might have negative consequences, such as immunosuppression and impaired growth performance.

In a study by Greco et al. [59], 42 samples of compound feed for broiler chickens were contaminated with ZEN (median 50 µg/kg). In a study on mycotoxins in compound feed for broiler chickens, ZEN was found in 1 out of 22 samples at a low concentration of 0.5 µg/kg [69]. Labuda et al. [65] detected ZEN in 88% (44) of the samples with an average concentration of 21 µg/kg and a maximum content of 86 µg/kg. Driehuis et al. [66] analysed 72 samples of compound feed, and ZEN was identified in 28% of the samples with an average of 80 µg/kg and a maximum level of 363 µg/kg. In the compound feed samples analysed by Martins et al. [61], 13% of the samples were found to be contaminated with ZEN at a level between 104 and 356 µg/kg. Zachariasova et al. [68] analysed a total of 70 broiler compound feed samples, and ZEN was found at a maximum content of 104 g/kg. In our study, it was observed that the average levels of ZEN contamination in broiler compound feed samples were lower than those obtained in the studies by Driehuis et al., Martins et al. and Zachariasova et al. [61,66,68].

In northern Romania, according to the observations of the authors involved in this study, as well as local and national sources of monitoring of meteorological phenomena in the period 2019–2023, the increased humidity of 2019–2021 is highlighted as being a result of heavy rains with increased periodicity, leading to the occurrence of average humidity levels ranging between 74 and 76% compared to 2022 and 2023, which recorded an average humidity of 65%.

The grains that can contribute to mycotoxin pollution in compound feed are mainly those that are susceptible to infestation by mycotoxin-producing moulds or fungi. The most commonly affected are the following: maize—a staple in feeds and often affected by fungi of the genera Fusarium, Aspergillus and Penicillium, which produce mycotoxins such as AFT, FUM and DON; wheat—also vulnerable to mycotoxin contamination, especially DON; barley and rye can be contaminated especially with DON and ZEN.

Meteorological factors could explain the higher contamination of feed raw materials and later the complete compound feed for broilers, as observed in the results obtained for the years 2019 and 2020. However, we have to consider that some mycotoxins such as AFT, although they prefer high humidity, have been confirmed by some studies to also accumulate during periods of prolonged drought [71,72]. The natural protective mechanisms

of forage plants are affected by long periods of drought, making it impossible for them to form protective structures against pathogens and thus to prevent the multiplication of mycotoxins.

Co-Occurrence

Co-occurrence in the case of compound feed is most often identified because the presence of mycotoxins in each of the raw materials used in the production of compound feed is very likely; therefore, current research is focused on the study of the synergistic or antagonistic effects of mycotoxins [10,73].

The data obtained in the current study highlight the co-occurrence of mycotoxins; thus, in 26% of the analysed samples, the concomitant presence of the five studied mycotoxins was confirmed. On the other hand, the combinations of AFT, FUM, OTA and AFT, FUM and ZEN were the least frequent, accounting for only 0.2% of cases in the compound feed samples analysed over the 5 years. In a 2019 investigation carried out in Spain, Arroyo-Manzanares et al. [74] demonstrated the co-occurrence of more than eight mycotoxins in 2.19% of pig compound feed samples; they also highlight that in 98.7% of the studied samples, they found at least two mycotoxins present simultaneously.

Also, in the case of the compound feed samples studied, we highlighted in Figure 1 that in most cases (38.51%) we identified the concomitant presence of four mycotoxins. Gruber Dominger et al. [75] explain that, in fact, the scenario of co-occurrence of mycotoxins in the feed is the most plausible.

5. Conclusions

Studies on the assessment of mycotoxin contamination in compound feed for broiler chickens in Romania are limited, and the high frequencies of contamination observed in this study emphasise the importance of improving mycotoxin control throughout the country. In the period 2019–2023, during which the analysed samples were collected from the combined feed factory in Romania, the samples showed contamination levels below the maximum limits allowed by the European Union; however, we consider it necessary to establish and apply biologically relevant regulatory thresholds and limits that could ensure the health of poultry. In addition, it has been shown that the distribution and presence of mycotoxins varied from one year to the next due to the changes in climatic conditions.

Co-occurrences of mycotoxins have been widely identified; even though levels have been low, there is a need to increase knowledge about their combined effects on animal and human health.

Concerning future research directions in Romania, these should be extended to more feed mills, feed materials and types of compound feed in order to develop a practical guide to provide a comprehensive overview of the risks in the food chain. In particular, it is also relevant to investigate the possibility of the transfer of mycotoxins into products of animal origin and to analyse the combined effects of mycotoxins on animal health, including synergistic or antagonistic effects between them.

Author Contributions: Conceptualisation, D.M.L. and I.M.P.; methodology, D.M.L. and S.-I.P.; software, D.M.L. and S.-I.P.; validation, D.M.L. and M.M.; formal analysis, D.M.L., C.-G.R.-R., S.-I.P. and I.M.P.; investigation, D.M.L., C.-G.R.-R. and M.M.; data curation, D.M.L. and I.M.P.; writing—original draft preparation, D.M.L. and S.-I.P.; writing—review and editing, D.M.L., S.-I.P., C.-G.R.-R. and I.M.P.; supervision, D.M.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data present in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Lorenzo, J.M.; Munekata, P.; Domínguez, R.; Pateiro, M.; Saraiva, J.; Franco Ruiz, D. Chapter 3—Main Groups of Microorganisms of Relevance for Food Safety and Stability: General Aspects and Overall Description. In *Innovative Technologies for Food Preservation: Inactivation of Spoilage and Pathogenic Microorganisms*; Academic Press: Cambridge, MA, USA, 2018; pp. 53–107.
2. Marin, D.E.; Pistol, G.C.; Procudin, C.V.; Taranu, I. Co-Contamination of Food and Feed with Mycotoxin and Bacteria and Possible Implications for Health. *Agriculture* **2022**, *12*, 1970. [CrossRef]
3. Pleadin, J.; Frece, J.; Markov, K. Mycotoxins in food and feed. *Adv. Food Nutr. Res.* **2019**, *89*, 297–345. [CrossRef]
4. Zeece, M. Food additives. In *Introduction to the Chemistry of Food*; Academic Press: Cambridge, MA, USA, 2020; pp. 251–311. [CrossRef]
5. Richard, J.L. Mycotoxins—An overview. In *Romer Labs' Guide to Mycotoxins*; Binder, E.M., Krska, R., Eds.; Anytime Publishing Services: Leicestershire, UK, 2015; pp. 1–48.
6. Marroquín-Cardona, A.G.; Johnson, N.M.; Phillips, T.D.; Hayes, A.W. Mycotoxins in a changing global environment—A review. *Food Chem. Toxicol.* **2014**, *69*, 220–230. [CrossRef]
7. Eskola, M.; Kos, G.; Elliott, C.T.; Hajšlová, J.; Mayar, S.; Krska, R. Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate' of 25%. *Crit. Rev. Food Sci. Nutr.* **2020**, *60*, 2773–2789. [CrossRef] [PubMed]
8. Alshannaq, A.; Yu, J.-H. Occurrence, Toxicity, and Analysis of Major Mycotoxins in Food. *Int. J. Environ. Res. Public Health* **2017**, *14*, 632. [CrossRef]
9. Anukul, N.; Vangnai, K.; Mahakarnchanakul, W. Significance of regulation limits in mycotoxin contamination in Asia and risk management programs at the national level. *J. Food Drug Anal.* **2013**, *21*, 227–241. [CrossRef]
10. Pinotti, L.; Ottoboni, M.; Giromini, C.; Dell'Orto, V.; Cheli, F. Mycotoxin Contamination in the EU Feed Supply Chain: A Focus on Cereal Byproducts. *Toxins* **2016**, *8*, 45. [CrossRef] [PubMed]
11. Matei, M.; Zaharia, R.; Petrescu, S.-I.; Radu-Rusu, C.G.; Simeanu, D.; Mierlita, D.; Pop, I.M. Persistent Organic Pollutants (POPs): A Review Focused on Occurrence and Incidence in Animal Feed and Cow Milk. *Agriculture* **2023**, *13*, 873. [CrossRef]
12. Probst, C.; Njapau, H.; Cotty, P.J. Outbreak of an acute aflatoxicosis in Kenya in 2004: Identification of the causal agent. *Appl. Environ. Microb.* **2007**, *73*, 2762–2764. [CrossRef] [PubMed]
13. Galarza-Seeber, R.; Latorre, J.D.; Bielke, L.R.; Kuttappan, V.A.; Wolfenden, A.D.; Hernandez-Velasco, X.; Merino-Guzman, R.; Vicente, J.L.; Donoghue, A.; Cross, D.; et al. Leaky gut and mycotoxins: Aflatoxin B1 does not increase gut permeability in broiler chickens. *Front. Vet. Sci.* **2016**, *3*, 10. [CrossRef] [PubMed]
14. D'Mello, J.P.E. Contaminants and toxins in animal feeds. In *Assessing Quality and Safety of Animal Feeds*; Jutzi, S., Ed.; Food and Agriculture Organization: Rome, Italy, 2004; Volume 160, pp. 107–128.
15. Commission Regulation (EU) No 574/2011 of 16 June 2011 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for nitrite, melamine, *Ambrosia* spp. and carry-over of certain coccidiostats and histomonostats and consolidating Annexes I and II thereto. *Off. J. Eur. Union* **2011**, *L159*, 7–24.
16. Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. *Off. J. Eur. Union* **2006**, *L229*, 7–9.
17. Reddy, K.R.N.; Salleh, B.; Saad, B.; Abbas, H.K.; Abel, C.A.; Shier, W.T. An overview of mycotoxin contamination in foods and its implications for human health. *Toxin Rev.* **2010**, *29*, 3–26. [CrossRef]
18. Ismail, A.; Gonçalves, B.L.; de Neeff, D.V.; Ponzilacqua, B.; Coppa, C.F.; Hintzsche, H.; Sajid, M.; Cruz, A.G.; Corassin, C.H.; Oliveira, C.A.F. Aflatoxin in foodstuffs: Occurrence and recent advances in decontamination. *Food Res. Int.* **2018**, *113*, 74–85. [CrossRef] [PubMed]
19. Rushing, B.R.; Selim, M.I. Aflatoxin B1: A review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. *Food Chem. Toxicol.* **2019**, *124*, 81–100. [CrossRef]
20. Guo, H.; Wang, P.; Liu, C.; Chang, J.; Yin, Q.; Wang, L.; Jin, S.; Zhu, Q.; Lu, F. Compound Mycotoxin Detoxifier Alleviating Aflatoxin B1 Toxic Effects on Broiler Growth Performance, Organ Damage and Gut Microbiota. *Poult. Sci.* **2023**, *102*, 102434. [CrossRef] [PubMed]
21. Paneru, D.; Sharma, M.K.; Shi, H.; Wang, J.; Kim, W.K. Aflatoxin B1 Impairs Bone Mineralization in Broiler Chickens. *Toxins* **2024**, *16*, 78. [CrossRef] [PubMed]
22. Sobrova, P.; Adam, V.; Vasatkova, A.; Beklova, M.; Zeman, L.; Kizek, R. Deoxynivalenol and its toxicity. *Interdiscip. Toxicol.* **2010**, *3*, 94–99. [CrossRef] [PubMed]

23. Wan, S.; Sun, N.; Li, H.; Khan, A.; Zheng, X.; Sun, Y.; Fan, R. Deoxynivalenol damages the intestinal barrier and biota of the broiler chickens. *BMC Vet. Res.* **2022**, *18*, 311. [CrossRef]
24. Peng, W.X.; Marchal, J.L.M.; Van der Poel, A.F.B. Strategies to prevent and reduce mycotoxins for compound feed manufacturing. *Anim. Feed. Sci. Technol.* **2018**, *237*, 129–153. [CrossRef]
25. Richard, J.L. Some major mycotoxins and their mycotoxicoses—An overview. *Int. J. Food Microbiol.* **2007**, *119*, 3–10. [CrossRef]
26. Anttila, A.; Bhat, R.V.; Bond, J.A.; Borghoff, S.J.; Bosch, F.X.; Carlson, G.P.; Castegnaro, M.; Cruzan, G.; Gelderblom, W.C.A.; Hass, U.; et al. *International Agency for Research on Cancer (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene*; IARC Press: Lyon, France, 2002; Volume 82, p. 367.
27. Völkel, I.; Schröer-Merker, E.; Czerny, C.P. The carry-over of mycotoxins in products of animal origin with special regard to its implications for the European food safety legislation. *Food Nutr. Sci.* **2011**, *2*, 852–867. [CrossRef]
28. El Khoury, A.; Atoui, A. Ochratoxin A: General overview and actual molecular status. *Toxins* **2010**, *2*, 461–493. [CrossRef] [PubMed]
29. World Health Organization (WHO); International Agency for Research on Cancer (IARC). *Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins*; IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans; IARC Publications: Lyon, France, 1993; p. 56.
30. Gaffoor, I.; Trail, F. Characterization of two polyketide synthase genes involved in zearalenone biosynthesis in *Gibberella zeae*. *Appl. Environ. Microbiol.* **2006**, *72*, 1793–1799. [CrossRef]
31. Pop, I.M.; Halga, P.; Avarvarei, T. Animal nutrition and feeding. In *Nutritia si Alimentatia Animalelor*; Tipo Moldova: Iasi, Romania, 2006.
32. European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain. Scientific Opinion on the risks for public health related to the presence of zearalenone in food. *EFSA J.* **2011**, *9*, 2197. [CrossRef]
33. Pop, I.M. Food quality and safety management. In *Managementul Calitatii si Sigurantei Alimentelor*; Tipo Moldova: Iasi, Romania, 2022.
34. Lapusneanu, D.M.; Simeanu, D.; Radu-Rusu, C.; Pop, C.; Postolache, A.N.; Pop, I.M. Case study on the prevention and control of mycotoxin contamination in a feed mill unit. *J. Hyg. Eng. Des. JHED* **2020**, *33*, 41–47.
35. Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 Laying down Requirements for Feed Hygiene. *Off. J. Eur. Union* **2005**, *L35*, 1–22.
36. Granados-Chinchilla, F.; Valenzuela-Martínez, C.; García-Murillo, B.; Aguilar-Madrigal, D.; Redondo-Solano, M.; Molina, A. Microbiological safety and presence of major mycotoxins in animal feed for laboratory animals in a developing country: The case of Costa Rica. *Animals* **2021**, *11*, 2389. [CrossRef]
37. Cegielska-Radziejewska, R.; Stuper-Szablewska, K.; Szablewski, T. Microflora and mycotoxin contamination in poultry feed mixtures from western Poland. *Ann. Agric. Environ. Med. AAEM* **2013**, *20*, 30–35.
38. Smith, M.-C.; Madec, S.; Coton, E.; Hymery, N. Natural Co-Occurrence of Mycotoxins in Foods and Feeds and Their in vitro Combined Toxicological Effects. *Toxins* **2016**, *8*, 94. [CrossRef]
39. *SR EN ISO 9001:2015*; Quality Management Systems—Requirements. Romanian Standards Association: Bucharest, Romania, 2015.
40. *SR EN ISO 22000:2019*; Food Safety Management Systems—Requirements for Any Organization in the Food Chain. Romanian Standards Association: Bucharest, Romania, 2019.
41. *SR ISO 45001:2018*; Occupational Health and Safety Management Systems—Requirements with Guidance for Use. Romanian Standards Association: Bucharest, Romania, 2018.
42. Rayat, C.S. Applications of Microsoft Excel in Statistical Methods. In *Statistical Methods in Medical Research*, 1st ed.; Rayat, C.S., Ed.; Springer: Singapore, 2018; pp. 139–146.
43. Abdolshahi, A.; Behdad Shokrollahi, Y. Chapter 2—Food contamination. In *Mycotoxins and Food Safety*; Suna, S., Ed.; IntechOpen: Rijeka, Croatia, 2020.
44. Guerre, P. Mycotoxin and Gut Microbiota Interactions. *Toxins* **2020**, *12*, 769. [CrossRef] [PubMed]
45. Stanley, D.; Bajagai, Y.S. Feed Safety and the Development of Poultry Intestinal Microbiota. *Animals* **2022**, *12*, 2890. [CrossRef] [PubMed]
46. Todd, E.C.D. Foodborne Diseases: Overview of Biological Hazards and Foodborne Diseases. In *Encyclopedia of Food Safety*; Academic Press: Cambridge, MA, USA, 2014; pp. 221–242. [CrossRef]
47. Cimbalo, A.; Alonso Garrido, M.; Font, G.; Manyes, L. Toxicity of mycotoxins in vivo on vertebrate organisms: A review. *Food Chem. Toxicol.* **2020**, *137*, 111161. [CrossRef] [PubMed]
48. Abrunhosa, L.; Morales, H.; Soares, C.; Calado, T.; Vila-Cha, A.S.; Pereira, M.; Venancio, A. A Review of Mycotoxins in Food and Feed Products in Portugal and Estimation of Probable Daily Intakes. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 249–265. [CrossRef] [PubMed]

49. Lăpușneanu, D.M.; Simeanu, D.; Radu-Rusu, C.-G.; Zaharia, R.; Pop, I.M. Microbiological Assessment of Broiler Compound Feed Production as Part of the Food Chain—A Case Study in a Romanian Feed Mill. *Agriculture* **2023**, *13*, 107. [CrossRef]
50. Khoshal, A.K.; Novak, B.; Martin, P.G.P.; Jenkins, T.; Neves, M.; Schatzmayr, G.; Oswald, I.P.; Pinton, P. Co-Occurrence of DON and Emerging Mycotoxins in Worldwide Finished Pig Feed and Their Combined Toxicity in Intestinal Cells. *Toxins* **2019**, *11*, 727. [CrossRef]
51. Shar, Z.H.; Shar, H.H.; Jatoi, A.; Sherazi, S.T.H.; Mahesar, S.A.; Khan, E.; Phanwar, Q.K. Natural co-occurrence of *Fusarium* toxins in poultry feed and its ingredients. *J. Consum. Prot. Food Saf.* **2020**, *15*, 341–350. [CrossRef]
52. Bennett, J.W.; Klich, M. Mycotoxins. *Clin. Microbiol. Rev.* **2003**, *16*, 497–516. [CrossRef] [PubMed]
53. Guerre, P. Worldwide Mycotoxins Exposure in Pig and Poultry Feed Formulations. *Toxins* **2016**, *8*, 350. [CrossRef]
54. Rawal, S.; Kim, J.E.; Coulombe, R., Jr. Aflatoxin B1 in poultry: Toxicology, metabolism and prevention. *Res. Vet. Sci.* **2010**, *89*, 325–331. [CrossRef] [PubMed]
55. Ochieng, P.E.; Scippo, M.; Kemboi, D.C.; Croubels, S.; Okoth, S.; Kang'ethe, E.K.; Doupovec, B.; Gathumbi, J.K.; Lindahl, J.F.; Antonissen, G. Mycotoxins in Poultry Feed and Feed Ingredients from Sub-Saharan Africa and Their Impact on the Production of Broiler and Layer Chickens: A Review. *Toxins* **2021**, *13*, 633. [CrossRef]
56. Alshannaq, A.; Yu, J.-H. Analysis of E.U. Rapid Alert System (RASFF) Notifications for Aflatoxins in Exported U.S. Food and Feed Products for 2010–2019. *Toxins* **2021**, *13*, 90. [CrossRef]
57. De Rijk, T.C.; Van Egmond, H.P.; Van der Fels-Klerx, H.J.; Herbes, R.; De Nijs, M.; Samson, R.A.; Slate, A.B.; Van der Spiegel, M. A study of the 2013 Western European issue of aflatoxin contamination of maize from the Balkan area. *World Mycotoxin J.* **2015**, *8*, 641–651. [CrossRef]
58. Kosicki, R.; Błajet-Kosicka, A.; Grajewski, J.; Twarużek, M. Multiannual mycotoxin survey in feed materials and feeding stuffs. *Anim. Feed Sci. Technol.* **2016**, *215*, 165–180. [CrossRef]
59. Greco, M.V.; Franchi, M.L.; Rico Golba, S.L.; Pardo, A.G.; Pose, G.N. Mycotoxins and mycotoxigenic fungi in poultry feed for food-producing animals. *Sci. World J.* **2014**, *2014*, 968215. [CrossRef] [PubMed]
60. Decastelli, L.; Lai, J.; Gramaglia, M.; Monaco, A.; Nachtmann, C.; Oldano, F.; Ruffer, M.; Sezian, A.; Bandirola, C. Aflatoxins occurrence in milk and feed in Northern Italy during 2004–2005. *Food Control* **2007**, *18*, 1263–1266. [CrossRef]
61. Martins, H.M.; Marques, M.; Almeida, I.; Guerra, M.M.; Bernardo, F. Mycotoxins in feedstuffs in Portugal: An overview. *Mycotoxin Res.* **2008**, *24*, 19–23. [CrossRef] [PubMed]
62. Šegvić Klarić, M.; Cvetnić, Z.; Pepeljnjak, S.; Kosalec, I. Co-occurrence of Aflatoxins, Ochratoxin A, Fumonisins, and Zearalenone in Cereals and Feed, Determined by Competitive Direct Enzyme-Linked Immunosorbent Assay and Thin-Layer Chromatography. *Arch. Ind. Hyg. Toxicol.* **2009**, *60*, 427–433. [CrossRef]
63. Mierlita, D.; Teușdea, A.C.; Matei, M.; Pascal, C.; Simeanu, D.; Pop, I.M. Effect of Dietary Incorporation of Hemp Seeds Alone or with Dried Fruit Pomace on Laying Hens' Performance and on Lipid Composition and Oxidation Status of Egg Yolks. *Animals* **2024**, *14*, 750. [CrossRef] [PubMed]
64. EFSA Panel on Contaminants in the Food Chain (CONTAM); Schrenk, D.; Bignami, M.; Bodin, L.; Chipman, J.K.; Del Mazo, J.; Grasl-Kraupp, B.; Hogstrand, C.; Hoogenboom, L.R.; Leblanc, J.C.; et al. Risk assessment of aflatoxins in food. *EFSA J.* **2020**, *18*, e06040. [CrossRef]
65. Labuda, R.; Parich, A.; Berthiller, F.; Tančinová, D. Incidence of trichothecenes and zearalenone in poultry feed mixtures from Slovakia. *Int. J. Food Microbiol.* **2005**, *105*, 19–25. [CrossRef] [PubMed]
66. Driehuis, F.; Spanjer, M.C.; Scholten, J.M.; Te Giffel, M.C. Occurrence of mycotoxins in feedstuffs of dairy cows and estimation of total dietary intakes. *J. Dairy Sci.* **2008**, *91*, 4261–4271. [CrossRef] [PubMed]
67. Almeida, I.; Martins, H.M.; Santos, S.; Costa, J.M.; Bernardo, F. Co-occurrence of mycotoxins in swine feed produced in Portugal. *Mycotoxin Res.* **2011**, *27*, 177–181. [CrossRef]
68. Zachariasova, M.; Dzuman, Z.; Veprikova, Z.; Hajkova, K.; Jiru, M.; Vaclavikova, M.; Zachariasova, A.; Pospichalova, M.; Florian, M.; Hajslova, J. Occurrence of multiple mycotoxins in European feedingstuffs, assessment of dietary intake by farm animals. *Anim. Feed Sci. Technol.* **2014**, *193*, 124–140. [CrossRef]
69. Jaimez, J.; Fente, C.A.; Franco, C.M.; Cepeda, A.; Vazquez, B.I. A survey of the fungal contamination and presence of ochratoxin A and zearalenone on Spanish feed and raw materials. *J. Sci. Food Agric.* **2004**, *84*, 832–840. [CrossRef]
70. Liu, J.; Applegate, T. Zearalenone (ZEN) in Livestock and Poultry: Dose, Toxicokinetics, Toxicity and Estrogenicity. *Toxins* **2020**, *12*, 377. [CrossRef] [PubMed]
71. Liu, C.; Van der Fels-Klerx, H.J. Quantitative Modeling of Climate Change Impacts on Mycotoxins in Cereals: A Review. *Toxins* **2021**, *13*, 276. [CrossRef]
72. Medina, A.; Gilbert, M.K.; Mack, B.M.; OBrian, G.R.; Rodríguez, A.; Bhatnagar, D.; Payne, G.; Magan, N. Interactions between water activity and temperature on the Aspergillus flavus transcriptome and aflatoxin B1 production. *Int. J. Food Microbiol.* **2017**, *256*, 36–44. [CrossRef] [PubMed]

73. Muñoz-Solano, B.; González-Peñas, E. Co-Occurrence of Mycotoxins in Feed for Cattle, Pigs, Poultry, and Sheep in Navarra, a Region of Northern Spain. *Toxins* **2023**, *15*, 172. [CrossRef] [PubMed]
74. Arroyo-Manzanares, N.; Rodríguez-Estévez, V.; Arenas-Fernández, P.; García-Campaña, A.M.; Gámiz-Gracia, L. Occurrence of Mycotoxins in Swine Feeding from Spain. *Toxins* **2019**, *11*, 342. [CrossRef]
75. Gruber-Dorninger, C.; Jenkins, T.; Schatzmayr, G. Global Mycotoxin Occurrence in Feed: A Ten-Year Survey. *Toxins* **2019**, *11*, 375. [CrossRef] [PubMed]