Journal of Agriculture and Food Research 24 (2025) 102399

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Toxicogenic fungi and mycotoxins seasonality in poultry farms: Implications for animal health and food safety

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ARTICLE INFO

Keywords:
Poultry production
Toxigenic fungi
Mycotoxins
Growth performance
Food safety

ABSTRACT

Poultry farms are hostpost for fungi and mycotoxins proliferation. Still, seasonal dynamics on of these hazards on farm and their impacts on animals/workers health, and lastly on food safety remain unexplored. This study provides the first comprehensive assessment of seasonal (summer/winter) fungal/mycotoxins exposure in poultry farms environment (Electrostatic dust cloths (EDC), feed, bed) and broiler chicken tissues (breast, liver) to identify potential exposure risks for broiler chickens/workers health and food safety. Viable mycotoxigenic fungi and mycotoxins profile (LC-MS/MS) was characterized and cytotoxicity assessment was performed using A549 (human alveolar epithelial) and SK (swine kidney) cells. Zootechnical parameters were also measured to evaluate broiler chickens' growth performance. Summer conditions favour the proliferation of mycotoxigenic fungi belonging to *Penicillium* and *Aspergillus* genera, where co-contamination of hazardous mycotoxins (ZEN/TRCs/FBs) was prevalent. Considering cytotoxicity assessment, EDC samples induce high toxicity in 47 % of A549 cells during summer, whereas on bed samples, high toxicity was obtained during winter on both cell lines (100 % SK cells; 83 % A549 cells). Our results evidence diverse exposure pathways (inhalation, dermal contact) and seasonal health risks. Regarding mycotoxins in biological samples, ZEN was detected in 53.3 % of liver samples, highlighting chronic exposure risks. Thus, future research should focus on toxicokinetics/toxicodynamics of co-occurring mycotoxins in animal production environments and their subsequent human exposure through the food chain. Given that climate change may exacerbate seasonal fungal/mycotoxin contamination, understanding these interactions is crucial for improving risk assessment frameworks and to implement protection measures on the farm level and along the food chain.

1. Introduction

Over the last decade, livestock production systems have intensified, with poultry meat being the most produced type of meat worldwide [1]. By 2024, poultry production has reached approximately 138.75 million metric tons, a significant rise from the 115 million metric tons in 2016 [2]. This trend, reflects shifts in consumers preferences and the industrialization of the poultry sector [1]. Recent statistics evidence poultry meat as the most consumed type of meat globally [2]. This tendency is also observed in Portugal, where poultry, particularly chicken, is the most widely consumed type of meat [3].

The poultry farm environment is highly conducive to microbial proliferation and the detection of pathogenic bacteria and zoonotic risks are well-documented [4–6]. In contrast, fungal contamination is poorly understood despite its dual threat to animal and human health [7]. Current research on fungal assessment has predominantly focused on animal feed, where *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. are the most significant genera [8]. These mycotoxigenic fungi, capable of producing harmful mycotoxins, thrive in diverse environments and under favourable conditions, synthesize mycotoxins that pose

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https://doi.org/10.1016/j.jafr.2025.102399
Received 28 July 2025; Received in revised form 19 September 2025; Accepted 27 September 2025
Available online 27 September 2025

significant risks to both humans and animals health [9,10].

Aflatoxins (AFs), fumonisins (FBs), ochratoxins (OTs), trichothecenes (TRCs) (HT-2, T-2 toxin, deoxynivalenol) and zearalenone (ZEN) [8] are the most agriculturally and toxicologically significant mycotoxins. Their negative effect on broilers' health has already been demonstrated, especially the effects of AFs on liver toxicity, TRCs in lower feed efficiency, and OTs in immunosuppression, increasing disease susceptibility [11–13]. Emerging mycotoxins (EMs) (eg.: moniliformin (MON), nivalenol (NIV), diacetoxyscirpenol (DAS), sterigmatocystin (STER), mycophenolic acid (MPA) are also gaining attention due to their increasing detection in crops and animal feed, lack of toxicological data and regulatory limits [14].

To date, most studies have shown the widespread prevalence of mycotoxins in poultry feed ingredients, which remains the primary source of animal exposure [8,13,15,16]. Animal feed is mainly composed by cereals and cereal-based products, which are major source of mycotoxins. Fungal contamination and consequently mycotoxins production can occur in the field before harvest or during storage and transportation, leading to mycotoxin accumulation in feed [17]. Since fungi can produce different mycotoxins simultaneously, the co-occurrence of different mycotoxins in raw ingredients and feeds is a frequent scenario worldwide [17].

Some studies explore mycotoxins effects on poultry health and performance [12,13]. Among the effects, mycotoxins impair broilers physiological performance by disrupting nutrient digestion and metabolism, while suppressing imune responses which increase susceptibility to a variety of co-infections [18,19].

Once in broilers body, these toxins are metabolized in the liver, kidneys, and digestive tract. If not excreted, they can accumulate in edible tissues [17]. Critical knowledge gaps remain concerning the bioaccumulation potential of these toxins into edible tissues (liver, kidney) and eggs ultimately contributing to human dietary exposure [11,17].

Mycotoxins exposure can also occur through inhalation. These secondary metabolites can become airborne by attaching to dust particles and fungal spores. These carriers facilitate their entry into the respiratory system, posing potential respiratory risks [20–22]. In poultry farms, dust comprises complex mixtures of microorganisms and their metabolites and animals are at risk of inhaling airborne mycotoxins [1,23]. This exposure can also extend to poultry workers, during certain operations [20–22]. Despite these recognized hazards, significant knowledge gaps remain on farm monitoring of these contaminants and their health risks to workers [22,24], animals [19,25] and food safety [16,18]. Also the influence of climate variations on their distribution [1,26] and the health effects of co-exposure to multiple mycotoxins remain limited [17, 23].

Risk characterization in poultry farms is particularly challenging, as workers and animals are simultaneously exposed to complex mixtures of chemicals, microorganisms, and their metabolites. Exposure assessments often follow a one-pollutant-at-a-time approach, which overlooks the potential health effects of co-exposure to mixtures In this context, cytotoxicity assessments offers advantages by preserving the overall biological impact of environmental samples [27].

In vitro studies represent an important first-line screening tool for characterizing the combined effects of multiple stressors [27,28]. Different cell lines may be used depending on the environment evaluated, the route of exposure, and the target cells of specific pollutants. For example, human alveolar epithelial (A549) cells, are frequently used to test the toxicity of airborne mixtures, whereas swine kidney (SK) cells are suitable for evaluating pollutants related to feed because they are extremely sensitive to mycotoxins. Despite their relevance, cytotoxicity studies remain few explored, specially in the context of poultry production [27]. Enhancing their use could reinforce the role of cytotoxicity assays in risk assessment and management by advancing our understanding of the links between co-exposure to environmental pollutants and adverse health outcomes [27,28].

Based on the identified knowledge gaps, this study aims to characterize the diversity of toxigenic fungi and mycotoxins in poultry farm environments across different seasons (summer and winter), using a multi-approach sampling strategy (environmental and biological samples). To evaluate potential health effects on animals and workers, this study incorporates cytotoxicity assessment. Cells lines were selected based on the most probable exposure routes and target cells of fungi and mycotoxins: Human alveolar epithelial cells were used to assess inhalation risks and swine kidney cells has a model for mycotoxins exposure due to their high sensitivity to these contaminants [27,28]. Additionally, to evaluate practical implications, broilers growth parameters were monitored and mycotoxins contamination in broilers edible tissues were evaluated to assess the impact on production efficiency and potential food safety hazards.

By integrating environmental/biological monitoring and seasonal analysis, this research contributes to a better understanding of fungal and mycotoxins exposure pathways relevant to both animal and occupational health and food safety.

2. Material and methods

2.1. Experimental design and data collection

This study is part of a large exploratory study aiming to assess microbial contamination and exposure risks in poultry farms. Fourteen poultry pavilions (PP) located in Madeira island, Portugal were assessed during winter (December–February) and summer (June–August) seasons. PP were prepared prior to birds arrival, by covering the floor with bedding material (wood shavings) which remains in place throughout the end of the production cycle (aprox. 35 days). Birds were fed with 3 types of feed during their growth. Electrostatic dust cloths (EDC, n = 78), feed samples (n = 76) and bed samples (n = 78) were collected weekly inside PP during the production cycle (3 weeks). Along with the zootechnical parameters of broiler chickens.

Following the summer production cycle, broilers breast (n = 15) and liver (n = 15) samples were provided by a licensed slaughterhouse (Fig. 1). Animals were not killed for the sole purpose of the experiment and the samples were obtained as by-products of the food chain.

2.2. Samples collection and extraction

Electrostatic dust cloths (Swiffer, Portugal) were positioned horizontally on a sanitized plate in the center of each poultry pavilion (PP) at a height of 1.5 m above the ground. Composite samples of broilers feed (10 g) were collected directly from feeders, while bedding material (10 g) was collected from random locations within each PP [29]. Following the production cycle, after slaughter, composite samples of broilers breast (10 g) and livers (10 g) were obtained [30]. All samples were placed in sterilized bags and kept refrigerated (0–4 °C) until laboratory processing and analysis [31].

For fungal characterization, samples were extracted following pre-established protocols [29,32]. Briefly, airborne settled dust collected in EDC was eluted with 20 mL 0.1 % Tween 80 saline (0.9 % NaCl) via orbital shaking (250 rpm, 30 min). Feed and bedding material samples were eluted in 90 mL 0.1 % Tween 80 saline (0.9 % NaCl). Breast and liver samples were homogenized in 90 mL 0.1 % Tween 80 saline (0.9 % NaCl) as performed elsewhere [33,34].

2.3. Characterization of viable mycobiota

Assuming PP as a highly contaminated environment [1], 150 μL of samples extracts (EDC,bed, feed) were inoculated into dichloran glycerol (DG18) agar-based in order to obtained a higher diversity of fungal genera and restrict the colony size of fast-growing fungal species [22, 35]. Breast and liver samples, were inoculated in malt extract agar (MEA) and dichloran-glycerol agar (DG18) media.

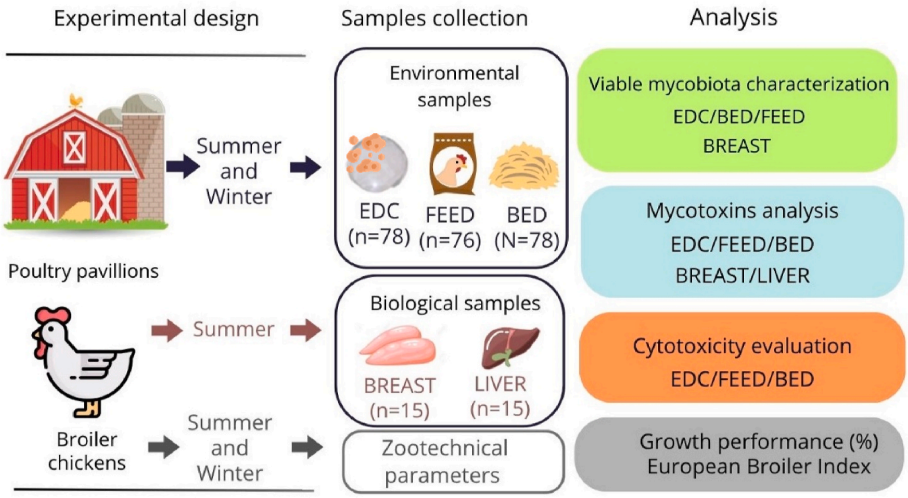

Fig. 1. Flowchart of experimental design and analysis conducted. *Electrostatic dust cloths (EDC)*.

All samples were incubated at 27 °C for 5–7 days [34]. Fungal identification was performed through macroscopic and microscopic examination [36], by an experienced mycologist and fungal concentrations were obtained (CFU/m^2/day; CFU/g). Biological samples (breast and liver) came from broilers raised on the same farms where environmental samples were collected. These samples reflect fungal contamination in by-products of the food chain, with both the farm environmentand the slaughterhouse serving as potential contamination sources [30,37].

2.4. Mycotoxins analysis

EDC (n = 78), bed (n = 78), feed (n = 76), broilers breast (n = 15) and liver (n = 15) were screened for mycotoxins presence. Sample processing and chromatographic analysis of mycotoxins were carried out in accordance with the protocol outlined in Viegas et al. [38]. Concerning samples preparation, EDC samples (0.05 g) were extracted with 2.5 ml of acetonitrile (ACN): water (H2O): acetic acid (AcOH) (79:20:1, v/v/v) solution for 60 min. The sample was then centrifuged (5 min, 5000 rpm) and 1.9 mL of the extract was evaporated under stream of nitrogen, reconstituted in 380 μL of methanol/water (2:8, v/v), and centrifuged again (30 min, 14500 rpm). Feed and bed samples (0.5 g) were extracted with 2.0 mL of ACN: H2O: AcOH (79:20:1, v/v/v) for 60 min. Following centrifugation (10 min, 7000 rpm), a 0.25 mL aliquot of the resulting supernatant was diluted with an equal volume (0.25 mL) of water, mixed on vortex, and subsequently centrifuged to prepare them for HPLC-MS/MS analysis.

For mycotoxin analysis, broiler breast and liver samples were processed using similar extraction and clean-up procedures, with slight modifications depending on the tissue type and target analytes. For each matrix, 3 g of tissue were homogenized in 12 mL of acetonitrile/water (8:2, v/v) for 3 min and centrifuged (10 min, 7000 rpm). Four milliliters of the supernatant were transferred into a 50 mL tube, followed by the addition of 20 μL of β-glucuronidase (37 °C, 18 h) to allow hydrolysis of possible conjugated metabolites, and 20 μL of an internal standard mixture specific for each matrix. After incubation, samples were evaporated to dryness under a nitrogen stream at 40 °C. The residue was reconstituted in 1 mL of methanol, subjected to sonication (3 min) and vortexing (5 min), and diluted with 25 mL of phosphate-buffered saline (PBS). The resulting extracts were passed through immunoaffinity columns connected in tandem, Aflatest® (Vicam, Watertown, USA) and DZT MS-PREP® (R-Biopharm, Glasgow, UK) for the determination of aflatoxins, trichothecenes, and zearalenone in liver, an Ochraprep and DZT MS-PREP (R-Biopharm, Flasgow, UK) for OTA, trichothecenes, and zearalenone in breast muscle. Mycotoxins were eluted with 1.5 mL of methanol/acetic acid (98:2, v/v), evaporated to dryness under nitrogen, and reconstituted in 150 μL of water/methanol (7:3, v/v) for HPLC–MS/MS analysis.

In liver samples, the following mycotoxins were analysed: aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2), zearalenone and its metabolites (α-zearalenol, β-zearalenol, α-zearalanol, β-zearalanol), deoxynivalenol, deepoxy-deoxynivalenol, T-2 toxin, and HT-2 toxin. The internal standards used were: ^{13}C-AFB_1 (25 ng/mL), ^{13}C-DON (500 ng/mL), ^{13}C-T_2 (100 ng/mL), ^{13}C-HT_2 (200 ng/mL), and ^{13}C-ZEN (50 ng/mL).

In breast samples, the mycotoxins analysed included ochratoxin A, zearalenone and its metabolites, DON, DOM-1, T-2, and HT-2. The internal standards used were: ^{13}C-OTA (10 ng/mL), ^{13}C-DON (500 ng/mL), ^{13}C-T_2 (1000 ng/mL), ^{13}C-HT_2 (200 ng/mL), and ^{13}C-ZEN (50 ng/mL).

The determination of mycotoxins was performed using a a Nexera high performance liquid chromatograph (HPLC) (Shimadzu, Tokyo, Japan) coupled with an 5500 QTrap mass spectrometer (Sciex, Foster City, CA, USA).

Separation of compounds from EDC, bed, and feed extracts was performed using a Gemini C18 chromatographic column (150 × 4.6 mm, 5 μm; Phenomenex, Torrance, CA, USA). The mobile phases consisted of (A) methanol/water/acetic acid (10:89:1, v/v/v) and (B) methanol/water/acetic acid (97:2:1, v/v/v), both containing 5 mmol/L ammonium acetate. The flow rate was set at 1.0 mL/min, with an injection volume of 5 μL, and the following gradient program was applied: 0–2.0 min, 0 % B; 2.0–5.0 min, linear increase to 50 % B; 5.0–14.0 min, linear increase to 100 % B; 14.0–18.0 min, hold at 100 % B; 18.0–22.5 min, return to 0 % B for re-equilibration.

For breast and liver extracts, separation was carried out on a Nucleodur C18 Gravity-SB column (150 × 2.0 mm, 3 μm; Macherey-Nagel, Düren, Germany). The mobile phases consisted of (A) water/acetic acid (99.9:0.1, v/v) and (B) methanol/acetic acid (99.9:0.1, v/v), both with 5 mmol/L ammonium acetate. The flow rate was 0.5 mL/min with an injection volume of 4 μL. The gradient program was as follows: 0.1–12 min, linear increase from 10 % to 95 % B; 12–14 min, hold at 95 % B; 14–20 min, return to 10 % B for column re-equilibration. Instrumental analysis and electrospray ionization (ESI)–MS/MS were performed by multiple reaction monitoring (MRM) in positive and negative ion modes as published before [39]. The specific transitions of precursor and product ions are presented in Table S1 – Supplementary material. The mycotoxins analysed, as well as their limits of detection (LOD) are presented in Table S2 – Supplementary material.

2.5. Cytotoxicity evaluation

EDC (n = 78), feed (n = 76) and beds (n = 78) samples were serial diluted (two-fold) and evaluated for effects on metabolic activity in A549 and SK cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay based on a pre-established protocol [32]. Cells were maintained in Minimum Essential Medium Eagle (MEM) (Sigma-Aldrich, St. Louis, MI, USA) supplemented with 10,000 units penicillin, 10 mg/mL streptomycin and fetal calf serum (FCS, Sigma-Aldrich, USA) in a CO_2-incubator (CB, BINDER GmbH, Tuttlingen, Germany) (5 % CO_2, 37 °C, 98 % humidity).

Cells were then detached from the vessels with 0.25 % (w/v) Trypsin 0.53 mM EDTA and resuspended in the culture medium. Their number was determined using a cell counter (Scepter™ 2.0 Cell Counter, Merck Millipore, Burlington, MA, USA). Subsequently, 2.5 x 10^5 cells were seeded per well of a 96-well microtiter plate. Cell suspensions (100 µL) were then incubated with the test samples in 96-well plate. After 48 h incubation (5 % CO_2, 37 °C, 98 % humidity), cell viability was determined through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay at 510 nm (ELISA LEDETECT 96, biomed Dr. Wieser GmbH; MikroWin 2013SC software) as described previously [40]. The dilution medium served as a negative control. The IC50 value, defined as the test concentration reducing metabolic activity to 50 % of control levels, was established as the toxicity threshold.

2.6. Broilers chickens growth performance

Growth performance parameters were evaluated following [41], and calculations were performed by using the formulas described in Table S3 – Supplementary material.

Zootechnical parameters were assessed by registering body weight (BW) on the 1st day and then on the day of feed change (2nd and 3rd week). Based on the differences on the BW recorded, the body weight gain (BWG), total body weight gain (TWG) were calculated for each period and the growth rate (%) was obtained (Eq.1- Table S3 Supplementary material). Feed intake (FI) was calculated from the differences between the initial added feed and feed residues at the end of each phase. Based on these data the average daily weight gain (ADBWG) and average daily feed intake (ADFI) were calculated (Eq. 2 and 3 – Table S3 Supplementary material). The feed conversion ratio (FCR) per 1 kg of body weight gain (FCR) was obtained (Eq.4 -Table S3 Supplementary material). Poultries mortality was registered and viability (V) percentage was calculated weekly (Eq.5 -Table S3 Supplementary material). European Production Efficiency Factor (EPEF) and European Broiler Index (EBI) were subsequently determined at the end of the production cycle (Eq. 6 and 1 respectively – Table S3 Supplementary material)

2.7. Statistical analysis

Statistical analyses were performed using SPSS statistical software, version 27.0 for Windows. Normality was assessed using the Shapiro–Wilk test. Fungal and mycotoxins contamination between seasons (summer and winter), within each matrix was compared using the Mann-Whitney-U test, since the normality assumption was not verified. Species diversity was assessed using Shannon and Simpson indices based on the formulas described in Viegas et al. [42]. Zootechnical parameters were expressed as mean ± standard error of the mean (SEM) for normally distributed data, and median [interquartile range (IQR) for non-normally distributed data. Seasonal differences were verified using independent samples t-tests (parametric) or Mann-Whitney *U* test (non-parametric). To study the relationship between fungal and mycotoxins contamination and zootechnical parameters, spearman's correlation coefficient was used, since the assumption of normality was not verified.

3. Results

3.1. Fungal and mycotoxins seasonality in the poultry pavilions

Fungal contamination differs significantly between seasons, on bed (u = 1904.5, p = 0.001) and EDC samples (u = 420, p = 0.001). On bed samples, the highest median fungal values were obtained during winter ($3.54x10^2$ CFU/g), while on EDC, the highest values were obtained during summer ($4.48x10^2$ CFU/m^2/day). Regarding feed samples, no significant seasonal differences were detected, though the highest fungal values were obtained during winter ($2.00x10^2$ CFU/g) (Fig. 2a).

For mycotoxins, contamination exhibits significant seasonal variation in all matrices namely, bed (u = 1407.5, p < 0.001), feed (u = 113, p < 0.001), and EDC (u = 1029.5, p = 0.003). Bed and feed evidence the highest values during winter ($1.51x10^2$ CFU/g; $1.01x10^2$ CFU/g). EDC evidence the highest contamination during summer ($3.50x10^2$ CFU/m^2/day) (Fig. 2b).

3.1.1. Fungal species diversity and toxigenic potential

Shannon (H) and Simpson (D) indices were used as standard metrics to characterize both the richness and evenness of microbial communities [43]. In EDC, fungal communities were richer (H) and taxonomically diverse (D) during winter (H = 2.04; D = 6.65) when compared to summer (H = 1.29, D = 2.50). In feed, higher fungal diversity was found during summer (H = 1.19; D = 2.14), while on winter fungal communites had lower diversity and higher species dominance (H = 0.74; D = 1.45). Similar values were obtained in bed samples (Summer:H = 1.44; D = 2.88; Winter:H = 0.61; D = 0.42) (Table S4 Supplementary material).

Concerning fungal distribution between seasons, the highest number of species was found during summer, in feed (11 species) and bed samples (10 species). Similar diversity was found between seasons in EDC (Winter: 11 species; Summer: 10 species). Whereas during winter, lower diversity was found in bed and feed samples (9 and 8 species, respectively). Among the fungal species identified, *Aspergillus* spp. and *Penicillium* spp. were the genera with the highest values in each matrix (Table 1). When comparing both seasons, the highest levels of *Aspergillus* spp. were obtained during summer in EDC (7.50–1545.45 CFU/m^2/day), feed (10–700 CFU/g), and bed (10–700 CFU/g) samples. Similar values were obtained for *Penicillium* spp. when comparing seasonal variation in EDC (winter: 7.58–415.58 CFU/m^2/day; summer: 12.99–2890.90 CFU/m^2/day^1), feed (winter: 7.58–415.58 CFU/g; summer: 10–2420 CFU/g) and bed (winter: 10–1650 CFU/g; summer: 10–2420 CFU/g) samples.

Mycotoxigenic species belonging to genus *Aspergillus* (sections *Flavi, Aspergilli, Circumdati, Fumigati, Nidulantes, Nigri, Restricti*), *Penicillium* spp. were prevalent in all matrices in both seasons.

3.1.2. Mycotoxins diversity and contamination levels

Mycotoxins were ubiquitously detected in feed samples collected from the feeders (100 % winter; 100 % summer), despite the shortest time within PP, when compared with bed (97.61 % winter; 70.51 % summer) and EDC (61.91 % winter; 72.22 % summer) samples.

Regarding mycotoxins diversity during winter, FBs were frequently detected (feed: 100 % FB1, FB2, FB3; bed: 92.90 % FB1, 83.30 % FB2; EDC: 64.3 % FB1; 47.60 % FB2). ROQ-C was common in bed (28.6 %) and feed (7.14 %). Other mycotoxins were exclusively found in each matrix such as MON and NIV in feed (70.70 %; 2.44 % respectively), MPA in bed (26.20 %) and STER on EDC (2.38 %). During summer ZEN was commonly detected among matrices (100 % feed; 59 % EDC; 6.62 % bed). FBs were also recurrent in feed (61.11 % FB1; 33.33 % FB2) and bed (68.90 % FB1) samples. DON was frequent among bed (97.10 %) and EDC (70 %) samples. TRCs (DON/DAS/T-2/HT-2) and ochratoxin A (OTA-A) were only detected in feed samples (100 %DON; 100 % DAS; 100 % T-2; 100 % HT-2; 5.56 % OTA-A). While ROQ-C, MPA and STER where exclusively detected in bed samples (36.90 %; 5.41 %; 3.38 %

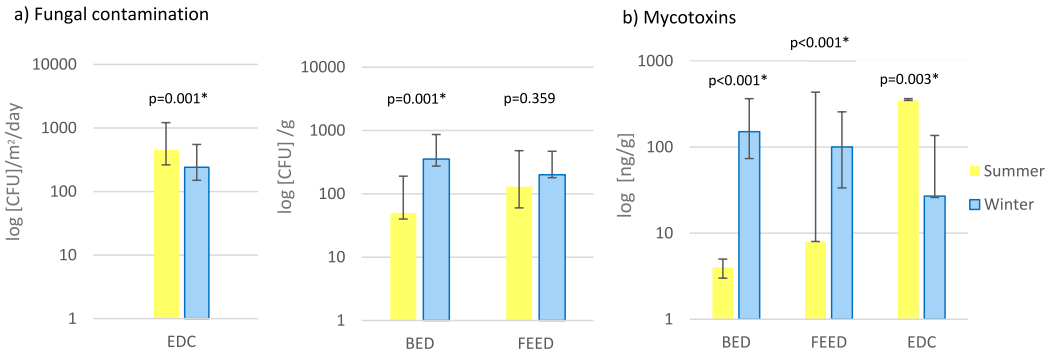

Fig. 2. Comparison of median values for a) fungal contamination (EDC -CFU/m^2/day; Bed/Feed - CFU/g) and b) mycotoxins (ng/g) between seasons (summer/winter) in poultry pavilions. Mann- Whitney test results. (*)Statistically significant differences at 5 % significance level.

Table 1
Fungal levels across matrices during winter and summer, and mycotoxigenic potential (MP) – ('+' -toxigenic; '-' not toxigenic).

	Winter			Summer		
	Range (CFU/m^2/day) (CFU/g)	Species	MP	Range (CFU/m^2/day) (CFU/g)	Species	MP
EDC	[9.09–831.17]	*Aspergillus* spp.[a]	+	[12.99–2890.90]	*Penicillium* spp.	+
	[7.58–415.58]	*Penicillium* sp	+	[7.50–1545.45]	*Aspergillus* spp. [b]	+
	[11.36–333.33]	*Paecilomyces* spp.	–	[12.98–116.88]	*Paecilomyces* spp.	–
FEED	[6–1540]	*Penicillium* spp.	+	[10–2420]	*Penicillium* spp.	+
	[1–210]	*Aspergillus* spp.[c]	+	[10−700]	*Aspergillus* spp.[d]	+
	[3–150]	*Mucor* spp.	-	[10−170]	*Paecilomyces* spp.	–
	[6–20]	*Paecilomyces* spp.	–	[10–60]	*Mucor* spp.	–
	[2]	*Fusarium solani*	+	[10]	*Chrysosporium* spp.	–
				[10]	*Syncephalastrum racemosum*	–
BED	[10–1650]	*Penicillium* spp.	+	[10–2420]	*Penicillium* spp.	+
	[10−650]	*Aspergillus* spp.[e]	+	[10−700]	*Aspergillus* spp.[f]	+
	[50]	*Mucor* spp.	–	[10−170]	*Paecilomyces* spp.	–
	[6–20]	*Paecilomyces* spp.	–	[10]	*Mucor* spp.	–

[a] Section Flavi, Aspergilli, Circumdati, Fumigati, Nidulantes, Nigri, Restricti.
[b] Section Nigri, Flavi, Nidulantes, Aspergilli, Fumigati, Circumdati, Restricti.
[c] Section Aspergilli, Flavi, Fumigati, Nidulantes, Nigri.
[d] Section Aspergilli, Circumdati, Flavi, Fumigati, Nidulantes, Nigri.
[e] Section Aspergilli, Flavi, Fumigati, Nidulantes, Nigri.
[f] Section Aspergilli, Circumdati, Flavi, Nidulantes.

respectively (Fig. 3).

Mycotoxins co-occurrence was frequent, with 80.60 % of samples containing at least two mycotoxins, with 78.01 % above the LOQ. Feed samples contained up to 5 simultaneous mycotoxins in both seasons. Seasonal variation was evident. In winter FBs/MON was a recurrent combination in feed samples and in summer ZEN/TRCs/FBs was frequently found. EDC and bed had the highest incidence of co-contamination during winter and FBs (FB1/FB2) were the most recurrent combination, whereas in summer, DON/ZEN and DOM/FB1 were frequently found in EDC and bed, respectively.

3.1.3. *Effect of the collected samples on cell viability*

The cytotoxicity of EDC, bed and feed samples was tested by exposing A549 and SK cells to samples' washed extracts. Cytotoxicity levels were defined based on samples dilution (Table 2). The majority of samples exhibited some level of toxicity on both cell lines. Higher toxicity levels were found during summer in EDC and A459 cells were more affected (47 % high toxicity) when compared to SK cells (19 % high toxicity). This was also observed in feed samples, where toxicity was greater during summer in A549 cells (50 % high toxicity) when compared to SK cells (6 % high toxicity). Notably, high toxicity was found during winter on bed samples in both cell lines (100 % SK cells; 83 % A549 cells) when compared to the prevalence of samples inducing high toxicity in SK cells (8 %) and A549 cells (47 %) during summer.

3.2. *Zootechnical data and broiler chickens' growth performance*

Zootechnical data were evaluated over the production cycle (35 days), and comparative analyses were conducted to assess seasonal variation in growth performance (Table 3).

Zootechnical parameters were significantly affected by seasonal variation, namely BW ($p = 0.039$), BWG ($P = 0.005$), ADBWG ($P = 0.005$) and growth rate ($p = 0.010$). Overall, broilers reared during winter evidence higher BW (median = 1947.5 g; IQR = 1925–2369 g), BWG (median = 1404.0 g; IQR = 1160–1478 g), ADBWG (76.08 g. day^{-1}; IQR = 80.25–95.45 g) values and GR (%) (median = 131.71 %; IQR = 114.93–134.96 %). No statistically significant differences were found in FI ($p = 0.932$), ADFI ($p = 0.932$) and FCR ($p = 0.193$) when comparing both seasons. Still, FCR was lower in winter (1.47 ± 0.04) when compared to summer (1.66 ± 0.14). Viability remain similar in both season (winter: 99.22 %; IQR = 98.18–99.86 %; summer: 99.59 %; IQR = 99.14–99.72 %)

When evaluating the production efficiency, both European Production Efficiency Factor (EPEF) and European Broiler Index (EBI) were significantly higher ($p = 0.003$), in broilers raised during winter (EPEF = 351.44; IQR = 336.10–368.80, EBI = 344.30; IQR = 329.77–361.14)

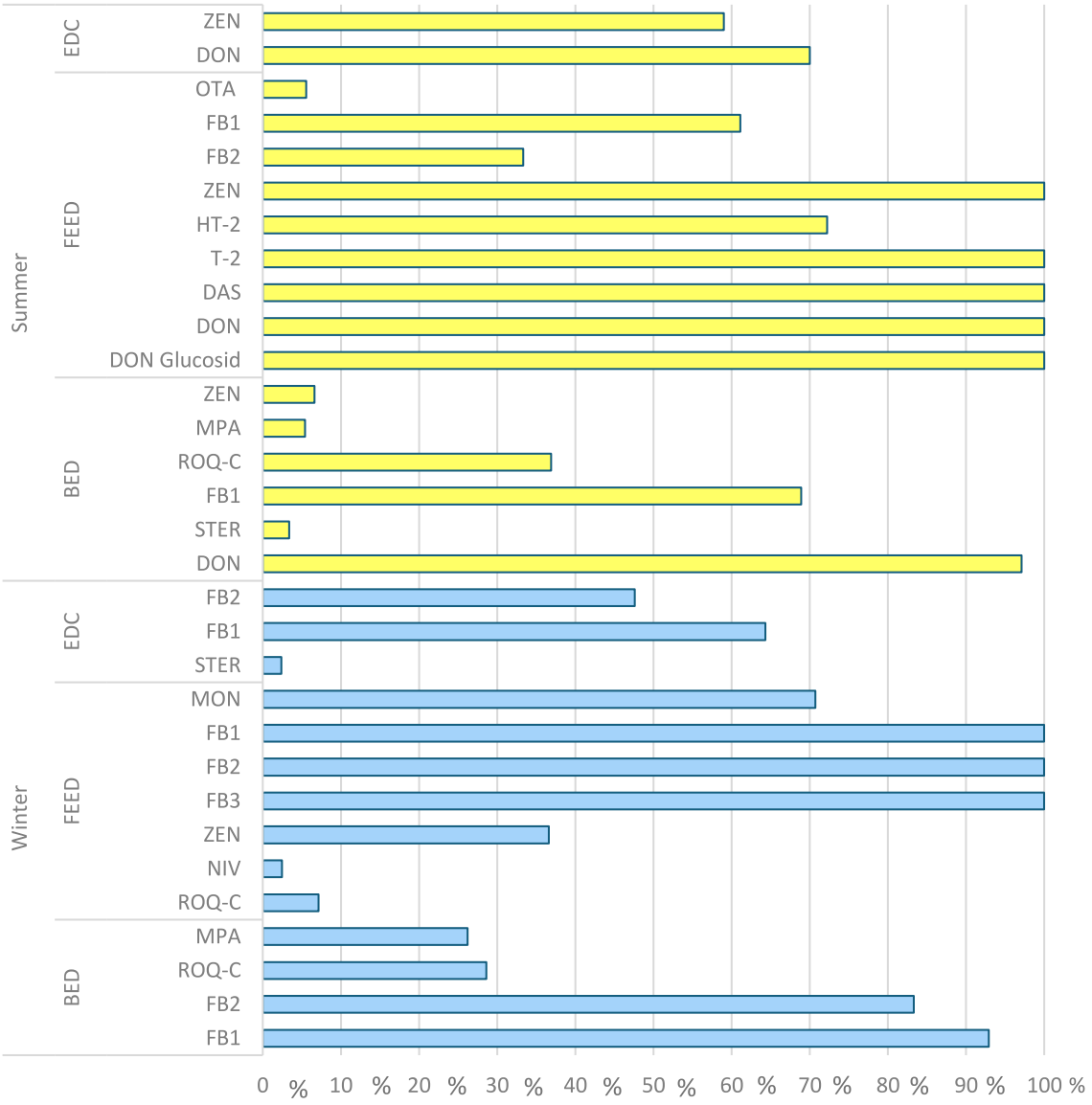

Fig. 3. Mycotoxins prevalence in environmental samples (EDC, Bed, Feed) during winter and summer. Zearalenone (ZEN), deepoxy-deoxynivalenol (DOM-1), ochratoxin A (OTA), fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 (FB3), toxin HT2 (HT-2), T-2 toxin T2 (T-2), diacetoxyscirpenol (DAS), Deoxynivalenol (DON), sterigmatocystin (STER), moniliformin (MON), nivalenol (NIV), roquefortine C (ROQ-C), mycophenolic acid (MPA).

Table 2
Toxicity level of EDC, feed and bed samples in SK and A549 cells (%, n samples).

		SK cells				A549 cells			
Matrice	Season	NC	Low	Medium	High	NC	Low	Medium	High
EDC	Winter (n = 42)	50 % (21)	50 % (21)	–	–	100 % (42)	–	–	–
	Summer (n = 36)	53 % (19)	22 % (8)	8 % (3)	19 % (6)	14 % (5)	31 % (11)	8 % (3)	47 % (17)
FEED	Winter (n = 40)	28 % (11)	68 % (27)	5 % (2)	–	38 % (15)	50 % (20)	13 % (5)	–
	Summer (n = 36)	8 % (3)	39 % (14)	47 % (17)	6 % (2)	19 % (7)	22 % (8)	8 % (3)	50 % (18)
BED	Winter (n = 42)	–	–	–	100 % (42)	7 % (3)	5 % (2)	5 % (2)	83 % (35)
	Summer (n = 36)	64 % (23)	8 % (3)	19 % (7)	8 % (3)	14 % (5)	31 % (11)	8 % (3)	47 % (17)

*A549, human alveolar epithelial cells; SK, swine kidney cells; EDC, Electrostatic dust collector; High, IC50≥3rd dilution; Moderate, IC50 at 2nd dilution; Low, IC50 at 1st dilution; (NC)- no cytotoxicity.

Table 3
Comparison of zootechnical parameters and performance of broiler chickens reared (35 days) during summer and winter. *Values are presented as: [a]median [Q1-Q3] for non-normally distributed data; [b] mean ± SEM for normally distributed data; Mann–Whitney U test for non-parametric data, and t-test for normally distributed data.*

	Values				p
	Winter		Summer		
BW (g)[a]	1947.5	[1925–2369]	1887.00	[1650–1952]	0.039*
BWG(g)[a]	1521.5	[1405–1909]	1404.00	[1160–1478]	0.005*
ADBWG(g)[a]	76.075	[80.25–95.45]	70.2	[58–73.90]	0.005*
GR (%)[a]	131.71	[114.93–134.96]	116.13	[108.41–118.48]	0.010*
FI (g)[a]	2237.41	[2453.57–2355.20]	2355.20	[1541.44–2731.46]	0.932
ADFI (g)[a]	111.87	[106.30–122.68]	117.76	[77.07–136.57]	0.932
FCR (kg/kg)[b]	1.47	±0.04	1.66	±0.14	0.193
Viability (%)[a]	99.22	[98.18–99.86]	99.59	[99.14–99.72]	0.932
EPEF[a]	351.44	[336.10–368.80]	238.82	[1541.44–2731.46]	0.003*
EBI[a]	344.30	[329.77–361.14]	230.11	[173.41–285.18]	0.003*

BW: body weight; BWG: body weight gain; ADBWG: average daily body weight gain; FI: feed intake; ADFI: average daily feed intake; FCR: feed conversion ratio; EPEF: European Production Efficiency Factor; EBI: European broiler index. *Statistically significant differences at the 5 % significance level.

compared to those in summer (EPEF = 238.82; IQR = 1541.44–2731.46, EBI = 230.11; IQR = 173.41–285.18).

3.3. Fungal and mycotoxins occurrence in poultry products

3.3.1. Fungal contamination in chicken breast

Fungal contamination had the highest values in DG18 (10–50 CFU/g) when compared to MEA values (10–50 CFU/g). Among the fungal species identified, *Aspergillus* spp. was prevalent on both culture media (80.95 % MEA; 91.89 % DG18). Lowest prevalence was obtained for *Penicillium* spp. (9.52 % MEA; 5.41 % DG18) and *Paecilomyces* spp. (1.35 % MEA; 4.76 % DG18). *Cladosporium* spp. and *Alternaria* spp. were found exclusively on DG18 (4.76 %) and MEA (1.35 %), respectively. Considering *Aspergillus* spp. diversity, 5 sections were identified on DG18 namely *section Nigri* (77.03 %), *Flavi* (6.76 %), *Fumigati* (5.41 %), *Nidulantes* (1.35 %), *Circumdati* (1.35 %). On MEA, 3 sections were recurrent, namely *Nigri* (61.90 %), *Fumigati* (9.52 %) and *Flavi* (9.52 %).

3.3.2. Mycotoxins detection in breast and liver

The selection of mycotoxins for breast and liver analysis was based on the fungal species identified in the poultry farm environment, particularly from the genera *Aspergillus, Penicillium*, and *Fusarium*, and their well-established capacity to produce aflatoxins, ochatoxin A, trichothecenes, and zearalenone. Priority was given to mycotoxins with documented hepatotoxic or growth-impairing effects in poultry and a known potential for carry-over into edible tissues. Breast muscle was chosen as the most consumed poultry product, while liver was included due to its susceptibility to mycotoxin accumulation and its relevance for assessing chronic dietary exposure [44]. ZEN was the only mycotoxin detected, being present in 8 out of 15 liver samples (53.3 %), all at concentrations below the limit of quantification (LOQ = 0.038 ng/g).

3.4. Correlation analysis

Correlations were performed between microbial contamination (fungi, mycotoxins), zootechnical parameters (BW, GR (%)) and cytotoxicity assay (SK and A549 cells) (Table 4). In EDC, higher fungal contamination relates to lower GR (%) ($r_S = -0.232$, $p = 0.041$) of broiler chickens and higher toxicity in A549 cells (rS = 0.369, p = 0.001). Higher mycotoxins contamination relates to higher BW ($r_S = 0.324$, $p = 0.004$) but lower GR (%) ($r_S = -0.564$, $p = 0.000$) and lower toxicity levels in SK ($r_S = -0.269$, $p = 0.017$) and A549 $r_S = -0.236$, $p = 0.038$) cells. In feed samples, higher fungal contamination relates to higher mycotoxins contamination ($r_S = 0.240$, $p = 0.037$). Whereas mycotoxins contamination relates to higher toxicity levels in SK ($r_S = 0.576$, $p = 0.000$) and A549 $r_S = 0.489$, $p = 0.000$). In bed samples, positive correlations were found between fungi and mycotoxins contamination ($r_S = 0.433$, $p = 0.000$). The same trend was observed between fungi and toxicity levels in SK cells ($r_S = 0.420$, $p = 0.000$). Mycotoxin levels correlated positively with toxicity levels on both cell lines (SK: $r_S = 708$, $p = 0.000$; A549: $r_S = 0{,}231$, $p = 0.042$)

Table 4
Study of the relationship between fungal and mycotoxins contamination in the different matrices (EDC,FEED, BED), zootechnical parameters (BW- body weight; GR (%)- growth rate), and cytotoxicity assessment (SK and A549 cells). Spearman correlation coefficient results.

		Microbial contamination	Zootechnical parameters		Cytotoxicity assessment	
Matrice		Mycotoxins	BW (g)	GR (%)	SK Cellls	A549Cells
EDC	Fungi	−0.002	0.034	−0.232*	0.053	0.369**
	Mycotoxins		0.324**	−0.564**	−0.269*	−0.236*
	BW (g)			−0.691**	−0.175	0.090
	GR (%)				0.109	−0.023
	SK Cellls					0.167
FEED	Fungi	0.240*	−0.080	0.092	−0.073	0.043
	Mycotoxins		0.138	−0.072	0.576**	0.489**
	BW (g)			−0.689**	−0.048	0.117
	GR (%)				0.149	−0.146
	SK Cellls					0.284*
BED	Fungi	0.433**	0.043	−0.163	0.420**	0.056
	Mycotoxins		−0.043	0.008	0.708**	0.231*
	BW (g)			−0.691**	−0.027	0.031
	GR (%)				−0.085	0.023
	SK Cellls					0.294**

*Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).a.

4. Discussion

Seasonal variations are known to significantly influence fungal contamination profiles [26,37]. However, its effects on fungal pathogens and their toxic compounds remain scarce [1,45]. These findings gain additional significance in the context of climate change, where fungal dissemination patterns are difficult to predict, potentially exacerbating exposure risks [46].

Considering fungal seasonality, fungal concentrations were higher in airborne settled dust (collected in EDC), during summer, driven by favourable climate conditions (optimal temperature) that promote fungal growth [47,48]. Additionally, lower relative humidity facilitates spore detachment from colonies, while increased airflows, typical for thermal optimization, enhance aerosolization and dispersion [49]. These factors collectively elevate inhalation exposure risks for both poultry and workers to fungal spores and mycotoxins [18].

Winter was identified as the highest risk season for fungal proliferation in broilers beds. This result was expected as visual inspections consistently confirmed moist bedding conditions throughout the production cycle. The elevated moisture content in wood shavings creates favourable microclimates for fungal proliferation [50]. In contrast to summer conditions, where broiler beds were relatively dry, likely due to higher ventilation and temperature [37].

Broiler chickens are very susceptible to temperature fluctuations, as high temperatures can induce heat stress, reduce feed intake and increase disease susceptibility [51]. While lower growth rates were observed during summer, feed intake remains similar between seasons. Notably, summer season also coincided with a higher concentration of mycotoxigenic fungi *(Penicillium* spp.*, Aspergillus* spp.*)*, which are considered potential agents of infection in animals and humans [9,29,52]. Despite the short production cycles, broiler chickens remain highly susceptible to fungal infections, particularly aspergillosis [48]. This is attributed to their unique respiratory tract, characterized by an "open" structure extending from the nasal cavity to abdominal organs. This facilitates rapid dissemination of inhaled fungal pathogens [37]. Besides, the respirable size of *Aspergillus* spp. spores (2–3 μm) facilitates their deposition in the lower respiratory tract, and subsequently infection [37].

Given the diversity of *Aspergillus* spp. and clinical importance, future studies should focus both on evaluating the contribution of *Aspergillus* sections isolates to the overall cytotoxicity, and characterizing mutations in section *Fumigati* isolates target [27,31].

Monitoring mycotoxins is vital for comprehensive microbial risk assessment, as fungal presence alone cannot confirm contamination [21,22,53]. In the accessed poultry farms, feed samples contained up to 5 mycotoxins simultaneously. Similarly, Liu et al. (2025) reported that over 70 % of poultry feed samples collected in China between 2021 and 2024 were contaminated with AFB1,DON and ZEA [54]. Similar results are reported in European countries, where mycotoxins are ubiquitously present in feed and feed raw materials [55].

This supports mycotoxins co-occurrence in feed formulations has a frequently reported scenario [14,15,17,56]. Moreover, co-contamination increases the risk for animal health due to additive and synergistic effects [54].

Our findings confirm *Fusarium* spp. mycotoxins (ZEN/TRCs/FBs) has frequent contaminants of poultry feed [15,19,57]. Corn, a major component of poultry feed, is particularly susceptible to contamination by *Fusarium* spp [58]. Since this grain is the main component of poultry feed formulations, their contamination by *Fusarium* spp. mycotoxins was expected [59].

A recent study in feed samples from 122 broiler farms in Kenya reported that ZEN, FBs, and DON were the most commonly occurring mycotoxins, with a co-occurrence probability exceeding 40 % [59]. Similar results were reported in a survey of 328 corn samples from the Southeastern U.S. Where 38.41 % of samples contained two mycotoxins simultaneously, namely ZEN, FBs, DON and AFB1 highlighting that mycotoxin contamination can also alter the nutrient profile of feed corn [58]. The high incidence of *Fusarium* spp. mycotoxins in these samples indicates that they were introduced into feed ingredients prior to harvest and processing by field toxigenic fungi [59]. This further supports contaminated feed has a source of mycotoxins in animal production [57,60].

In Europe, the prevalence of *Fusarium* species is rather common in cereals, with FBs being the most prevalent mycotoxins [55,61]. These contaminants are typically associated with temperate climates, and rising temperatures are expected to increase their prevalence. A similar trend is expected to occur for DON and ZEN in northern Europe [61]. As warmer temperatures and higher humidity in these regions, will favour fungal growth and mycotoxins production [61]. Thus, our findings support the idea that warmer temperatures and higher humidity enhance the risk of mycotoxin contamination [25,61].

In terms of toxicity, mycotoxins are well recognized for inducing a wide range of pathologies (mycotoxicoses) through chronic, low-dose exposure in both animals and humans [14,57]. Our results demonstrate co-exposure of regulated mycotoxins (OTA, FB1/FB2/FB3, DON, HT-2/T-2, ZEN) and emerging mycotoxins (MON, DAS, STER, NIV, MPA, ROQ-C) [14] within poultry facilities. Although, *Aspergillus* spp. (section *Flavi*) were prevalent during summer, no aflatoxins were detected in liver samples, in which contrast with previous studies reporting their presence [13,23,44]. This absence may reflect the effectiveness of strict EU regulations on maximum permitted levels of aflatoxins in feed (Directive 2002/32/EC) and the resulting control measures. Importantly, these findings highlight that the mere presence of potentially aflatoxigenic fungi does not necessarily result in detectable residues in edible tissues, reinforcing the need to combine fungal identification with direct mycotoxin measurements for accurate food safety risk assessment.

Diets contaminated with FBs are known to cause hepatotoxicity and growth impairment in most animal species [44]. TRCs can induce genotoxic and cytotoxic effects. Also, research indicates that dietary exposure to ZEN reduces BWG [44]. Notably, ZEN interaction with multi-mycotoxins (eg. AFB1,DON), exacerbates negatives effects, compromising production efficiency, the immune system and organ function of broilers (liver, intestinal barrier) [44,57]. These combined effects may have contributed to the lower broiler production efficiency during summer.

Poultry farm environments comprise complex mixtures with diverse contaminants (biological, chemical) [1]. The cytotoxicity assessment conducted in this study enables to evaluate the combined toxicity of these contaminants [27,62]. This approach more accurately reflects the real exposure scenario for both animals and workers [27].

Exposure risks vary depending on the season, with summer evidencing high toxicity risks, especially through inhalation (A549 cells). Toxicity is also influenced by matrix with bed samples exhibiting the highest toxicity on both cell lines, especially during winter. These results evidence seasonal health risks (increased respiratory risks during summer).

Fungi and toxins may arise from different sources (poor quality bedding, contaminated feed, dusty conditions) [1,7,52]. Exposure can occur by inhalation or by dermal contact, resulting in exposure by ingestion or through absorption. Considering these observations, key preventive measures to minimize exposure risks for both workers and animals should comprise the selection of high-quality feed and supplies, improved management of bedding material and adoption of good agricultural practices throughout the entire food chain (starting with the primary production of feed ingredients) [60].

Particular attention should be given to preventing mycotoxin transfer into food products. Once in broilers body, mycotoxins undergo biotransformation in the liver, and can accumulate in edible tissues (muscle/liver) [18,57]. Therefore, consumers may be indirectly exposed to hazardous substances through residual contamination in foods from animals fed with contaminated feeds [63].

Evidence of mycotoxin contamination in chicken meat or meat products is limited, with most studies focusing on AFs and OTs [16,34,64]. Nevertheless, feed contamination levels have been directly associated with AFB1 residues in poultry tissues, particularly in the liver [16,64]. Consequently, risk management strategies mainly focus on controlling plant-based food and feed to minimize mycotoxin transfer through the food chain [63].

In this study, mycotoxins were only detected in broilers liver, supporting the view that liver is the primary site of mycotoxin accumulation when compared to muscle tissue, as also observed by Lešić et al. (2025) [16,65].

Our results evidence ZEN in poultry liver samples. The role of ZEN in the disruption of the reproductive function in both humans and livestock is well-established [17,18,65]. Currently, ZEN is classified as non-carcinogenic (Group 3) by the International Agency for Research on Cancer (IARC). However, based on new evidence from animal and mechanistic studies, potential carcinogenic effects are suggested, warranting a high-priority re-evaluation, as highlighted by IARC Monographs [66]. Therefore, ensuring the safety of poultry meat remains critical, especially given its high demand and nutritional importance for children [16].

Climate change has intensified food safety risks by increasing mycotoxin contamination in crops. Mycotoxins can also transfer into animal derived products, raising further risks regarding human exposure through the food chain [10,25]. This poses serious health threats, especially to vulnerable groups like infants and young children, whose unique physiological traits (faster metabolic rates and higher food consumption relative to body weight), contribute for their increased exposure to contaminants. Also, their limited ability to detoxify these toxins further exacerbates exposure risks [18].

Other concerns are related to the prevalence of *Aspergillus* spp., potential producer of AFs/OTA/STER, in poultry meat samples obtained from the slaughterhouse, as thermal processing and industrial treatments may not full eliminate fungi and their mycotoxins, posing additional risks for chronic human exposure [18].

Fungal competitive dynamics are predicted to change due to climate change, favoring thermotolerant species like *Aspergillus* spp. As a result, aflatoxigenic strains are predicted to become more dominant, enhancing the possibility of aflatoxin contamination in agricultural products and endangering animal health and food safety [67].

As mentioned in previous studies [13,68,69], mycotoxins co-exposure can lead to synergistic or antagonistic effects during toxicokinetic and toxicodynamic phases However, data on these complex interactions, their combined effects on biological processes, and the carry-over rate to poultry animals remain poorly characterized and require further clarification.

5. Conclusion

This study evidence that seasonal conditions significantly influence fungal and mycotoxins contamination in poultry production environment. Summer conditions favour the proliferation of mycotoxigenic fungi and mycotoxins revealing significant risk to the respiratory health of animal and workers. Winter conditions promote fungal proliferation in broilers beds due to moisture content, highlighting distinct seasonal risks for fungi/mycotoxin exposure and the need of targeted management strategies.

Feed quality plays a central role in introducing mycotoxins into poultry farms with co-contamination of ZEN/TRCs/FBs during summer posing additional risks due to their combined toxicity. Mycotoxins accumulation in broilers liver underscores potential exposure risks through the food chain emphasizing the importance of feed quality control. Also, the persistence of toxigenic fungi (*Aspergillus*, *Penicillium*) in meat after industrial treatment reveals gaps in current mitigation protocols, often failing to eliminate fungal spores or mycotoxins.

Given the growing impact of climate change on fungal dynamics and mycotoxin production future research should focus on toxicokinetic/toxicodynamics of co-occurring mycotoxins ZEN/TRCs/FBs due to their synergistic potential and evaluate their long-term effect on broilers and workers. Understanding these interactions is crucial for improving risk assessment frameworks and ensure more effective protection measures on the farm level and along the food chain.

CRediT authorship contribution statement

Bianca Gomes: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Marta Dias:** Methodology. **Renata Cervantes:** Methodology. **Pedro Pena:** Methodology. **Magdalena Twarużek:** Writing – review & editing, Methodology. **Robert Kosicki:** Writing – review & editing, Methodology. **Jan Grajewski:** Writing – review & editing. **Elisabete Carolino:** Writing – review & editing, Validation, Software, Methodology. **Susana Viegas:** Writing – review & editing, Validation. **Carla Viegas:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work is supported by FCT – Fundação para a Ciência e Tecnologia, I.P. by the project UI/BD/153746/2022 (https://doi.org/10.54499/UI/BD/153746/2022) and CE3C unit UIDB/00329/2020 (https://doi.org/10.54499/UIDB/00329/2020); It is also supported by FCT/MCTES UIDP/05608/2020 (https://doi.org/10.54499/UIDP/05608/2020) and UIDB/05608/2020 (https://doi.org/10.54499/UIDB/05608/2020; 2023.01366.BD; 2023.01366.BD,https://doi.org/10.54499/2023.01366.BD); UI/BD/151431/2021(UI/BD/151431/2021″ title = "https://doi.org/10.54499/UI/BD/151431/2021">https://doi.org/10.54499/UI/BD/151431/2021) and also by Instituto Politécnico de Lisboa, Lisbon projects IPL/IDI&CA2024/WWTPSValor_ESTeSL and IPL/IDI&CA2024/MycoSOS_ESTeSL.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2025.102399.

Data availability

Data will be made available on request.

References

[1] B. Gomes, M. Dias, R. Cervantes, P. Pena, J. Santos, M. Vasconcelos Pinto, et al., One health approach to tackle microbial contamination on poultries—a systematic review, Toxics 11 (2023) 374.

[2] M. Shahbandeh, Meat consumption worldwide from 1990 to 2023, by meat type [Internet]. Statista: 2024 [cited 2025 June 25]. Available from: https://www.statista.com/statistics/274522/global-per-capita-consumption-of-meat/.

[3] R.P.F. Guiné, A. Gonçalves, E.T. Lemos, Food habits and knowledge related with meat on a sample of Portuguese consumers, in: The 2nd International Electronic Conference on Foods—"Future Foods and Food Technologies for a Sustainable World" MDPI, 2021, p. 2 [cited 2025 June 5], https://www.mdpi.com/2673-9976/6/1/2.

[4] A. Banerjee, R. Bardhan, M. Chowdhury, S.N. Joardar, D.P. Isore, K. Batabyal, et al., Characterization of beta-lactamase and biofilm producing *Enterobacteriaceae* isolated from organized and backyard farm ducks, Lett. Appl. Microbiol. 69 (2019) 110–115.

[5] I. Patuzzi, M. Orsini, V. Cibin, S. Petrin, E. Mastrorilli, A. Tiengo, et al., The interplay between Campylobacter and the caecal microbial community of commercial broiler chickens over time, Microorganisms 9 (2021) 221.

[6] M. Peng, D. Biswas, Environmental influences of high-density agricultural animal operation on human Forearm Skin Microflora, Microorganisms 8 (2020) 1481.
[7] B. Gomes, P. Pena, R. Cervantes, M. Dias, C. Viegas, Microbial contamination of bedding material: one health in poultry production, Int. J. Environ. Res. Public. Health 19 (2022) 16508.
[8] N. Nasaruddin, S. Jinap, N.I. Samsudin, N.H. Kamarulzaman, M. Sanny, Prevalence of mycotoxigenic fungi and assessment of aflatoxin contamination: a multiple case study along the integrated corn-based poultry feed supply chain in Malaysia, J. Sci. Food Agric. 101 (2021) 1812–1821.
[9] N.T. Mhlongo, M. Tekere, T. Sibanda, Prevalence and public health implications of mycotoxigenic fungi in treated drinking water systems, J. Water Health 17 (2019) 517–531.
[10] A. Moretti, M. Pascale, A.F. Logrieco, Mycotoxin risks under a climate change scenario in Europe, Trends Food Sci. Technol. 84 (2019) 38–40.
[11] C.G. Awuchi, E.N. Ondari, S. Nwozo, G.A. Odongo, I.J. Eseoghene, H. Twinomuhwezi, et al., Mycotoxins' toxicological Mechanisms involving humans, livestock and their associated health concerns: a review, Toxins 14 (2022) 167.
[12] L.M. Gómez-Osorio, M. Vasiljevic, J. Raj, J.J. Chaparro-Gutierréz, S. López-Osorio, Mycotoxins and coccidiosis in poultry – co-occurrence, interaction, and effects, Front. Vet. Sci. 11 (2024) 1387856.
[13] G.R. Murugesan, D.R. Ledoux, K. Naehrer, F. Berthiller, T.J. Applegate, B. Grenier, et al., Prevalence and effects of mycotoxins on poultry health and performance, and recent development in mycotoxin counteracting strategies, Poult. Sci. 94 (2015) 1298–1315.
[14] O. Kolawole, W. Siri-Anusornsak, A. Petchkongkaew, C. Elliott, A systematic review of global occurrence of emerging mycotoxins in crops and animal feeds, and their toxicity in livestock, Emerging Contam. 10 (2024) 100305.
[15] M.V. Greco, M.L. Franchi, S.L. Rico Golba, A.G. Pardo, G.N. Pose, Mycotoxins and mycotoxigenic fungi in poultry feed for food-producing animals, Sci. World J. 2014 (2014) 1–9.
[16] T. Lešić, J. Pleadin, N. Kudumija, D. Tomašković, A. Vulić, Mycotoxin residues in chicken breast muscle and liver, Foods 14 (2025) 2017.
[17] J. Tolosa, Y. Rodríguez-Carrasco, M.J. Ruiz, P. Vila-Donat, Multi-mycotoxin occurrence in feed, metabolism and carry-over to animal-derived food products: a review, Food Chem. Toxicol. 158 (2021) 112661.
[18] R.M. Olariu, N.I. Fiț, C.M. Bouari, G.C. Nadăș, Mycotoxins in broiler production: impacts on growth, Immunity, Vaccine Efficacy, and food safety, Toxins 17 (2025) 261.
[19] P.E. Ochieng, M.L. Scippo, D.C. Kemboi, S. Croubels, S. Okoth, E.K. Kang'ethe, et al., 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 13 (2021) 633.
[20] A.M. Marcelloni, D. Pigini, A. Chiominto, A. Gioffrè, E. Paba, Exposure to airborne mycotoxins: the riskiest working environments and tasks. Ann. Work Expo, Health 68 (2024) 19–35.
[21] S. Viegas, C. Viegas, A. Oppliger, Occupational exposure to mycotoxins: current knowledge and Prospects. Ann. Work expo, Health 62 (2018) 923–941.
[22] S. Viegas, C. Viegas, C. Martins, R. Assunção, Occupational exposure to mycotoxins—different sampling strategies telling a common story regarding occupational studies performed in Portugal (2012–2020), Toxins 12 (2020) 513.
[23] S. Viegas, L. Veiga, P. Figueredo, A. Almeida, E. Carolino, R. Sabino, et al., Occupational exposure to aflatoxin B1: the case of poultry and swine production, World Mycotoxin J. 6 (2013) 309–315.
[24] J. Skóra, K. Matusiak, P. Wojewódzki, A. Nowak, M. Sulyok, A. Ligocka, et al., Evaluation of microbiological and chemical contaminants in poultry farms, Int. J. Environ. Res. Public. Health 13 (2016) 192.
[25] N. El Darra, N. Grimi, I.A. Watson, A. El Khoury, Editorial: risk assessment of mycotoxins in food, Front. Nutr. [Internet] 10 (2023) [cited 2025 July 14]. Available from: https://www.frontiersin.org/articles/10.3389/fnut.2023.1145998/full.
[26] R.S. Chhaya, J. O'Brien, E. Cummins, Feed to fork risk assessment of mycotoxins under climate change influences - recent developments, Trends Food Sci. Technol. 126 (2022) 126–141.
[27] C. Viegas, P. Pena, B. Gomes, M. Dias, L. Aranha Caetano, S. Viegas, Are in vitro cytotoxicity assessments of environmental samples useful for characterizing the risk of exposure to multiple contaminants at the Workplace? A systematic review, Toxics 10 (2022) 72.
[28] M. Twarużek, E. Zastempowska, E. Soszczyńska, I. Ałtyn, The use of in vitro assays for the assessment 795 of cytotoxicity on the example of MTT test, Acta Univ. Lodz. Folia Biol. Oecologica (2018) 23–32.
[29] C. Viegas, E. Carolino, J. Malta-Vacas, R. Sabino, S. Viegas, C. Veríssimo, Fungal contamination of poultry litter: a public health Problem, J. Toxicol. Environ. Health A 75 (2012) 1341–1350.
[30] D. Althaus, C. Zweifel, R. Stephan, Analysis of a poultry slaughter process: influence of process stages on the microbiological contamination of broiler carcasses, Ital. J. Food Saf. [Internet] 6 (2017) [cited 2025 May 31];Available from: http://www.pagepressjournals.org/index.php/ijfs/article/view/7097.
[31] C. Viegas, B. Gomes, R. Pimenta, M. Dias, R. Cervantes, L.A. Caetano, et al., Microbial contamination in firefighter Headquarters': a neglected occupational exposure scenario, Build. Environ. 213 (2022) 108862.
[32] M. Dias, B. Gomes, P. Pena, R. Cervantes, S. Gonçalves, E. Carolino, et al., Assessment of the microbial contamination in "Do it Yourself" (DIY) stores - a holistic approach to protect workers' and consumers' health, Front. Public Health 12 (2024) 1483281.
[33] J hyun Kim, D.G. Yim, Assessment of the microbial level for livestock products in retail meat shops implementing HACCP system, Korean J. Food Sci. Anim. Resour. 36 (2016) 594–600.
[34] A.E. Morshdy, A.A. Abdel Samie, A.E. Tharwat, I.M. Elshorbagy, M.A. Hussein, Prevalence of mould in chicken meat-cuts, giblets and products with immunoaffinity detection of aflatoxin residues, Food Res. 5 (2021) 303–309.
[35] C. Viegas, M. Dias, E. Carolino, R. Sabino, Culture media and sampling collection method for Aspergillus spp. assessment: tackling the gap between recommendations and the scientific evidence, Atmosphere 12 (2020) 23.
[36] D. Hoog, J. Guarro, G. Gene, M. Figueiras, Atlas of Clinical Fungi—The Ultimate Benchtool for Diagnosis, first ed., Utr Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, 2016.
[37] G. Chen, D. Ma, Q. Huang, W. Tang, M. Wei, Y. Li, et al., Aerosol concentrations and fungal communities within broiler houses in different broiler growth stages in summer, Front. Vet. Sci. 8 (2021) 775502.
[38] C. Viegas, M. Dias, B. Almeida, L. Aranha Caetano, E. Carolino, A. Quintal Gomes, et al., Are workers from waste sorting industry really protected by wearing Filtering Respiratory Protective Devices? The gap between the myth and reality, Waste Manag. 102 (2020) 856–867.
[39] C. Viegas, C. Peixoto, B. Gomes, M. Dias, R. Cervantes, P. Pena, et al., Assessment of Portuguese fitness centers: bridging the knowledge gap on harmful microbial contamination with focus on fungi, Environ. Pollut. 350 (2024) 123976.
[40] M. Hanelt, M. Gareis, B. Kollarczik, Cytotoxicity of mycotoxins evaluated by the MTT-cell culture assay, Mycopathologia 128 (1994) 167–174.
[41] J. Biesek, M. Banaszak, M. Grabowicz, S. Wlaźlak, Chopped straw and coffee husks affect bedding chemical composition and the performance and foot pad condition of broiler chickens, Sci. Rep. 13 (2023) 6600.
[42] C. Viegas, B. Gomes, R. Cervantes, S. Moreira, M. Dias, P. Pena, et al., Microbial contamination in grocery stores from Portugal and Spain — the neglected indoor environment to be tackled in the scope of the One Health approach, Sci. Total Environ. 875 (2023) 162602.
[43] R.L. Górny, M. Gołofit-Szymczak, M. Cyprowski, A. Ławniczek-Wałczyk, A. Stobnicka, L.A. Wolska, Poultry house as point source of intense bioaerosol emission, Ann. Agric. Environ. Med. 30 (2023) 432–454.
[44] H. Okasha, B. Song, Z. Song, Hidden hazards revealed: mycotoxins and their masked Forms in poultry, Toxins 16 (2024) 137.
[45] G. Korfanty, E. Heifetz, J. Xu, Assessing thermal adaptation of a global sample of Aspergillus fumigatus: implications for climate change effects, Front. Public Health 11 (2023) 1059238.
[46] R. Assunção, C. Martins, S. Viegas, C. Viegas, L.S. Jakobsen, S. Pires, et al., Climate change and the health impact of aflatoxins exposure in Portugal – an overview, Food Addit. Contam. Part A 35 (2018) 1610–1621.
[47] K. Plewa, E. Lone, Seasonal biodiversity of pathogenic fungi in farming air area, Case study (2011).
[48] L.K. Wingfield, A. Phaichamnan, T. Jumnongnit, Evaluation of biosecurity and fungal contaminants in the poultry farms in Songkhla Province, Thailand, Sci. Asia 47 (2021) 486.
[49] X. Li, D. Liu, J. Yao, Aerosolization of fungal spores in indoor environments, Sci. Total Environ. 820 (2022) 153003.
[50] M.T. Munir, C. Belloncle, M. Irle, M. Federighi, Wood-based litter in poultry production: a review, Worlds Poult. Sci. J. 75 (2019) 5–16.
[51] R.L. Jallailudeen, I. Umar, B. John, M. Sarki, I. Hussaini, The effects of seasonal temperature variations on growth performance and mortality rates of broilers in Commercial and backyard poultry farms in maiduguri, Nigeria, J. Vet. Med. Sci. [Internet] (2024), https://doi.org/10.61148/JVMS/003.
[52] P. Arné, S. Thierry, D. Wang, M. Deville, Loc'h G. Le, A. Desoutter, et al., *Aspergillus fumigatus* in poultry, Int. J. Microbiol. 2011 (2011) 1–14.
[53] C. Viegas, B. Gomes, R. Pimenta, M. Dias, R. Cervantes, L.A. Caetano, et al., Microbial contamination in firefighter Headquarters': a neglected occupational exposure scenario, Build. Environ. 213 (2022) 108862.
[54] M. Liu, Z. Xia, Y. Zhang, R. Yang, W. Luo, L. Guo, et al., Contamination of aflatoxin B1, deoxynivalenol and zearalenone in feeds in China from 2021 to 2024, J. Anim. Sci. Biotechnol. 16 (2025) 66.
[55] E. Streit, G. Schatzmayr, P. Tassis, E. Tzika, D. Marin, I. Taranu, et al., Current situation of mycotoxin contamination and Co-occurrence in animal feed—focus on Europe, Toxins 4 (2012) 788–809.
[56] E. Khalifa, M.T. Mohesien, M.I. Mossa, M. Piekutowska, A.M. Alsuhaibani, B. A. Abdel-Wahab, et al., Diversity of toxigenic fungi in livestock and poultry feedstuffs, Int. J. Environ. Res. Public. Health 19 (2022) 7250.
[57] F. De Paul Tatfo Keutchatang, I. Sandrine Bouelet Ntsama, Keutchatang P. Richard, Mycotoxins in chicken farming: what you need to know [internet], in: M. Razzaghi-Abyaneh, M. Shams-Ghahfarokhi, M. Rai (Eds.), Research on Mycotoxins - from Mycotoxigenic Fungi to Innovative Strategies of Diagnosis, Control and Detoxification, IntechOpen, 2024 [cited 2025 June 5]. Available from: https://www.intechopen.com/chapters/1207145.
[58] A. Pokoo-Aikins, C.M. McDonough, T.R. Mitchell, J.A. Hawkins, L.F. Adams, Q. D. Read, et al., Mycotoxin contamination and the nutritional content of corn targeted for animal feed, Poult. Sci. 103 (2024) 104303.
[59] J.K. Njaramba, D.M. Muloi, M.V. Velde, S.D. Saeger, E.L. Ibayi, A. Moodley, et al., Multi-mycotoxin occurrence and their risk to poultry health in semi-intensive broiler farms in Kenya, Poult. Sci. 104 (2025) 105008.
[60] S. Viegas, R. Assunção, M. Twarużek, R. Kosicki, J. Grajewski, C. Viegas, Mycotoxins feed contamination in a dairy farm – potential implications for milk contamination and workers' exposure in a One Health approach, J. Sci. Food Agric. 100 (2020) 1118–1123.

[61] A. Casu, M. Camardo Leggieri, P. Toscano, P. Battilani, Changing climate, shifting mycotoxins: a comprehensive review of climate change impact on mycotoxin contamination, Compr. Rev. Food Sci. Food Saf. [Internet] 23 (2024) [cited 2025 July 14];Available from: https://ift.onlinelibrary.wiley.com/doi/10.1111/1541-4337.13323.

[62] M. Dias, B. Gomes, P. Pena, R. Cervantes, M. Rodriguez, B. Riesenberger, et al., Boosting knowledge on occupational exposure to microbial contamination in Portuguese carpentries, Front. Public Health 13 (2025) 1574881.

[63] V. Hort, M. Nicolas, A. Travel, C. Jondreville, C. Maleix, E. Baéza, et al., Carry-over assessment of fumonisins and zearalenone to poultry tissues after exposure of chickens to a contaminated diet – a study implementing stable-isotope dilution assay and UHPLC-MS/MS, Food Control 107 (2020) 106789.

[64] A. Sineque, C. Macuamule, F. Dos Anjos, Aflatoxin B1 contamination in chicken livers and gizzards from industrial and small abattoirs, measured by ELISA technique in maputo, Mozambique, Int. J. Environ. Res. Public. Health 14 (2017) 951.

[65] J. Xu, S. Li, L. Jiang, X. Gao, W. Liu, X. Zhu, et al., Baicalin protects against zearalenone-induced chicks liver and kidney injury by inhibiting expression of oxidative stress, inflammatory cytokines and caspase signaling pathway, Int. Immunopharmacol. 100 (2021) 108097.

[66] IARC, Report of the Advisory Group to Recommend Priorities for the IARC Monographs during 2025–2029, 2024. Lyon, France.

[67] M.E. Dimitrakopoulou, G. Marinos, M. Karvounis, G. Stoitsis, N. Manouselis, C. Thanopoulos, et al., What lies behind mycotoxin presence in animal feed? A case study, J. Food Protect. 88 (2025) 100464.

[68] S. Kulcsár, B. Kövesi, K. Balogh, E. Zándoki, Z. Ancsin, M. Erdélyi, et al., The Co-occurrence of T-2 toxin, deoxynivalenol, and fumonisin B1 activated the glutathione redox system in the EU-limiting doses in laying hens, Toxins 15 (2023) 305.

[69] C. Ladeira, G. Gajski, M. Meneses, M. Gerić, S. Viegas, The genotoxicity of an organic solvent mixture: a human biomonitoring study and translation of a real-scenario exposure to in vitro, Regul. Toxicol. Pharmacol. 116 (2020) 104726.