



REVIEW ARTICLE

Aflatoxins and co-occurring mycotoxins in poultry feed chains (2020–25): A systematic review of occurrence, toxicity, monitoring and mitigation

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Abstract

Aflatoxins, particularly aflatoxin B1 (AFB1) produced by *Aspergillus flavus* and *Aspergillus parasiticus*, remain persistent contaminants of maize- and groundnut-derived ingredients used in poultry feed, reducing bird productivity and creating consumer exposure through residues in eggs and edible tissues. We systematically searched PubMed, Scopus, ScienceDirect and Google Scholar for peer-reviewed primary studies published from January 2020 to December 2025. About 78 studies met the eligibility criteria; secondary sources were used only for contextual interpretation and were excluded from the PRISMA evidence count. Because the study designs, exposure matrices, analytical methods and outcome measures were highly heterogeneous, the evidence was synthesised using structured quantitative summaries and narrative comparison rather than statistical pooling. Across regions, AFB1 was the predominant aflatoxin and frequently co-occurred with deoxynivalenol, zearalenone, ochratoxin A and fumonisins. Mechanistic evidence linked aflatoxicosis to cytochrome P450-mediated bioactivation, oxidative and mitochondrial injury, immune dysregulation and intestinal-barrier disruption with mixture studies indicating additive or synergistic toxicity under some exposure conditions. Aluminosilicate clays and yeast-derived products showed the most consistent evidence of mitigation, although nutrient interactions, product variability, long-term safety and cost require consideration. Probiotics, synbiotics, enzymes and phytochemicals provided complementary protection, whereas nanomaterials, plasma-activated water and postbiotics require further safety, scale-up and regulatory evaluation. Rapid immunoassays are suitable for routine screening, while liquid chromatography-tandem mass spectrometry (LC-MS/MS) remains essential for confirmatory multi-mycotoxin analysis. Integrated prevention, risk-based surveillance and context-specific multimodal mitigation are therefore required across poultry-feed chains.

Keywords: aflatoxin B1; co-occurring mycotoxins; liquid chromatography-tandem mass spectrometry; mitigation; poultry feed; surveillance

Introduction

Mycotoxins are bioactive secondary metabolites produced by filamentous fungi that routinely contaminate food and feed supply chains, leading to economic losses and posing risks to animal and human health (1, 2). Among them, aflatoxins are of particular concern; aflatoxin B1 (AFB1) is classified as a Group 1 human carcinogen and is strongly associated with hepatocellular carcinoma, while chronic dietary exposure has also been linked to impaired growth and immune dysfunction in high-burden populations (1–3). The principal producers, *Aspergillus flavus* and *Aspergillus parasiticus*, thrive under warm and humid conditions common in tropical and subtropical regions (4, 5).

Modern poultry production is particularly vulnerable because rations rely heavily on maize, groundnut cake and other plant-derived ingredients susceptible to *Aspergillus* growth and aflatoxin accumulation when drying, storage and lot segregation are suboptimal (6–8). In poultry, contaminated feed reduces growth and feed efficiency, impairs carcass and egg quality, suppresses

immunity and may increase mortality (8–11). AFB1 can also be metabolised to aflatoxin M1 (AFM1) and other residues that are detectable in eggs and edible tissues, thereby linking feed contamination with consumer exposure (11–13).

Contemporary surveillance continues to identify AFB1 above commonly used guidance values in poultry feed, e.g., 20 µg/kg AFB1 and frequent co-occurrence with deoxynivalenol (DON), zearalenone (ZEA), ochratoxin A (OTA) and fumonisins (6, 7, 14–19). Mixtures complicate risk management because toxicological interactions vary with dose, duration and biological endpoint, while mitigation tools differ in toxin specificity. Persistent contamination is reinforced by uneven regulatory enforcement, limited access to confirmatory testing and practical constraints in sampling, drying, storage and segregation, especially in low- and middle-income settings (20–22).

Accordingly, this systematic review evaluates five interconnected domains: (i) the occurrence and co-occurrence of aflatoxins and other mycotoxins in poultry-feed chains; (ii) AFB1

toxicity and mechanistic interactions relevant to monitoring; (iii) mitigation strategies; (iv) analytical detection and tiered surveillance and (v) public-health and One Health implications. In addition to narrative synthesis, the review provides structured quantitative summaries of the 2020–25 evidence base and explicitly identifies areas in which heterogeneity prevents defensible statistical pooling.

Methods

Search strategy

A structured literature search was conducted using PubMed, Scopus, ScienceDirect and Google Scholar for peer-reviewed records published from 1st January 2020 to 31st December 2025. Search concepts covered aflatoxins and co-occurring mycotoxins; poultry species and production systems; feed, feed ingredients and poultry products; toxicological and biomarker outcomes; mitigation interventions and analytical monitoring methods. Boolean operators, phrase searching and truncation were adapted to each database. Reference lists of eligible primary studies and relevant reviews were hand-searched for additional records. Database-specific search strings, coverage dates and record yields are provided in Supplementary Table 1. Reviews, systematic reviews, book chapters, reports and risk evaluations were retained only for contextual interpretation and citation chasing and were excluded from the PRISMA evidence set.

Deduplication and screening

All retrieved records were compiled in a structured spreadsheet. Duplicate records were identified by matching digital object identifiers or PubMed identifiers, exact or near-exact titles, first authors and publication years, followed by manual verification. Two reviewers independently screened titles and abstracts and assessed potentially eligible full texts; disagreements were resolved by discussion. At title/abstract screening, records were excluded mainly because they were outside poultry or poultry-feed value chains, did not report aflatoxin or relevant co-occurring mycotoxin outcomes, were not primary research or did not evaluate an eligible occurrence, toxicological, mitigation, residue or analytical outcome.

Eligibility and data extraction

The present study included peer-reviewed primary monitoring or surveillance studies, experimental poultry toxicology studies, mitigation or intervention trials, upstream control studies directly relevant to poultry-feed ingredients and analytical validation or applied monitoring studies published during 2020–25. Eligible studies reported aflatoxins and/or co-occurring mycotoxins in poultry feed, feed ingredients, poultry products or relevant poultry systems, or evaluated a mitigation or monitoring outcome applicable to poultry-feed chains. The study excluded reviews, systematic reviews or meta-analyses, book chapters, reports or monographs, editorials, conference abstracts, non-peer-reviewed publications, studies outside the defined period, studies without aflatoxin-relevant outcomes and studies lacking sufficient extractable data. Extracted variables included country or region, matrix or biological system, study design, poultry type, mycotoxin profile, analytical method, exposure or intervention conditions, contamination measures, performance outcomes, biomarkers, histopathology and residues, as applicable.

Protocol registration and data synthesis

The review protocol was not prospectively registered. Eligibility criteria, data-extraction fields and synthesis domains were defined before final full-text extraction; the absence of prospective registration is acknowledged as a methodological limitation. Formal meta-analysis was not undertaken because the studies differed substantially in matrices, sampling frames, exposure concentrations and durations, analytical methods, mitigation formulations and outcome definitions. Structured quantitative summaries and narrative synthesis were therefore used. Each eligible study was assigned to one dominant study-purpose category according to its principal outcome.

Study selection

The searches yielded 2890 records: Scopus ($n = 297$), PubMed ($n = 333$), ScienceDirect ($n = 900$) and Google Scholar ($n = 1290$). After removal of 1850 duplicate records, 1040 unique records were screened and 810 were excluded for the reasons described above. Full texts were sought for 230 reports; 25 could not be retrieved, leaving 205 reports for eligibility assessment. A total of 127 reports were excluded because they were outside the poultry/feed context, lacked aflatoxin-relevant data, were secondary or non-peer-reviewed publications or had inadequate data or an ineligible design. Seventy-eight peer-reviewed primary studies were included in the systematic evidence synthesis (Fig. 1). Supplementary Table 2 contains only these eligible primary studies; secondary sources are cited solely for context.

Structured quantitative summary

Among the 78 eligible primary studies, 25 primarily addressed occurrence, surveillance, residues or knowledge-attitude-practice outcomes; 32 were *in vivo* mitigation or intervention studies; seven were *in vivo* toxicological or mechanistic studies; and 14 examined *in vitro* detoxification, analytical methods, molecular mechanisms or upstream crop/fungal control. These categories describe the dominant purpose of each study and do not imply mutually exclusive biological scope. The uneven distribution of designs, matrices and outcomes precluded a defensible pooled estimate; therefore, contamination ranges, direction-of-effect patterns, study counts and evidence limitations are reported.

Study quality appraisal

Given the diversity of designs, a qualitative risk-of-bias appraisal was applied rather than a single numerical score. Surveillance studies were assessed for sampling representativeness, sample size, analytical specificity, multi-mycotoxin coverage, detection limits and quality-control reporting. Experimental studies were assessed for exposure characterisation, randomisation, controls, co-exposures, intervention definition and completeness of clinical, biochemical, histopathological and performance outcomes. Analytical studies were appraised for validation characteristics and matrix applicability. Studies were not excluded solely because of risk of bias, but findings from small, single-site, single-dose or incompletely validated studies were interpreted cautiously.

Occurrence and surveillance in poultry-feed chains

Africa

Evidence from African countries suggests that aflatoxin contamination remains widespread in poultry-feed chains. Surveys from Uganda, Ghana, Nigeria and Senegal reported frequent detection and, in several datasets, concentrations above 20 µg/kg,

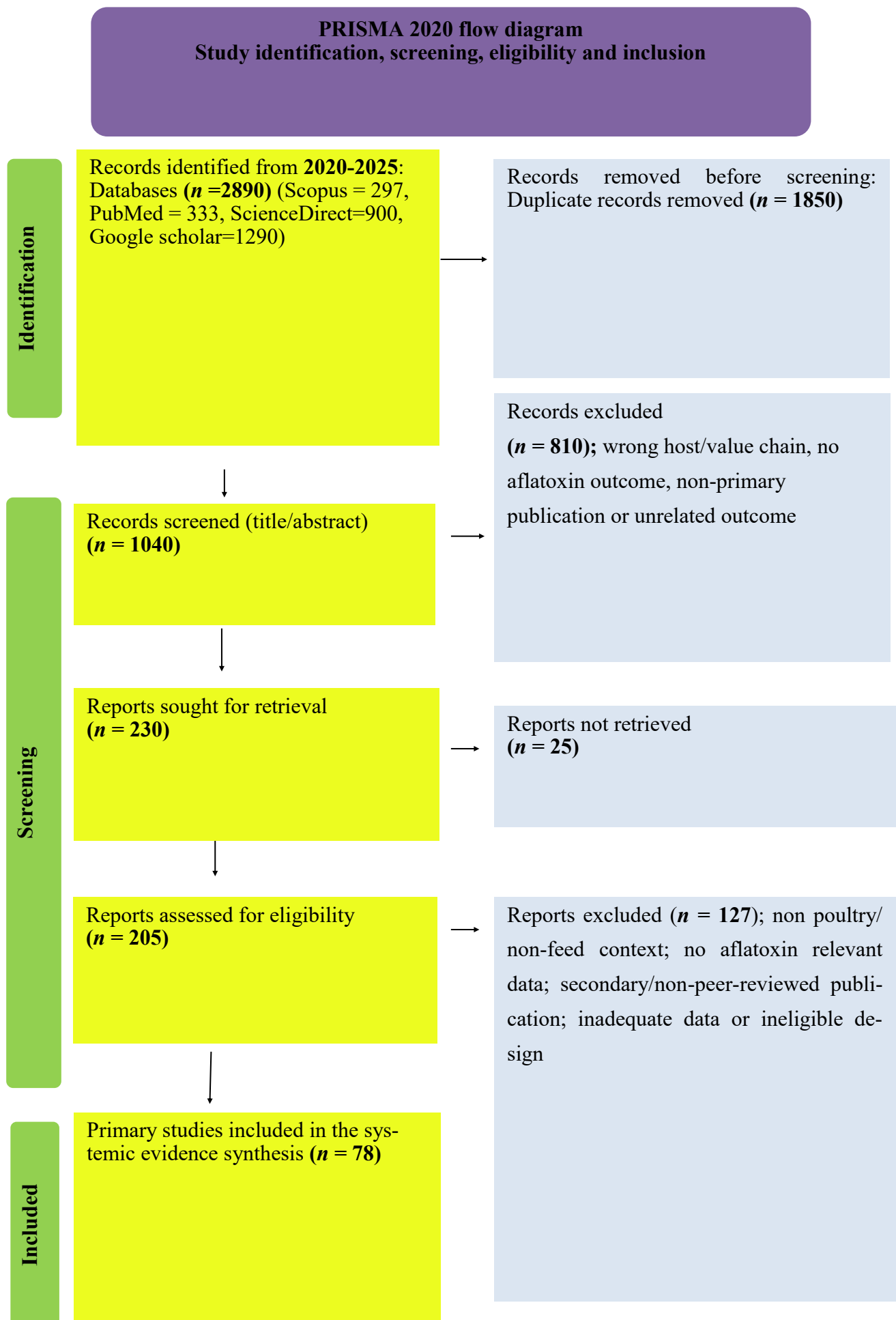


Fig. 1. PRISMA 2020 flow diagram of study identification, screening, eligibility and inclusion. Records identified from PubMed, Scopus, ScienceDirect and Google Scholar (2020–25). Screening decisions were made independently by two reviewers.

while an Ethiopian study identified major awareness and feed-handling gaps among poultry farmers (6, 14, 15, 20, 23, 24). Maize-derived ingredients were repeatedly identified as high-risk matrices, although study designs and analytical platforms varied.

Asia

Asian studies documented both AFB1 contamination and multi-mycotoxin co-occurrence. Farm and ingredient surveys in Pakistan and Iran quantified aflatoxins in poultry feeds, while Indian studies described combined AFB1-OTA exposure and associated field pathology (16, 17, 25, 26). Large Chinese datasets showed co-occurrence of AFB1, DON and ZEA and experimental work linked AFB1 exposure with impaired growth, altered serum biochemistry and oxidative stress (7, 11).

Middle East and North Africa

Studies from the Middle East and North Africa (MENA) highlight both significant aflatoxin contamination and emerging mitigation strategies. Residue surveys in Jordan detected AFB1 and other mycotoxins in eggs and edible tissues, while studies from Saudi Arabia identified aflatoxins, aflatoxigenic fungi and multi-mycotoxin contamination in compound feeds (12, 13, 19). Broiler trials evaluated hydrated sodium calcium aluminosilicate (HSCAS), date-pit adsorbents and other detoxifiers (27, 28). In Iraq, plasma-activated water was examined as a feed-treatment approach; whose reactive oxygen and nitrogen species can oxidatively transform mycotoxin structures under controlled conditions (29).

Europe

European evidence confirms that multi-mycotoxin exposure is not confined to tropical systems. A Spanish survey identified co-occurrence of AFB1, DON, OTA and ZEA and feed-mycobiota studies reported diverse *Aspergillus* and *Fusarium* populations; other European evidence linked mycotoxin exposure with poorer broiler productivity and vaccine responses (10, 18, 30).

Regional interpretation

Direct statistical ranking of regional contamination burdens was not undertaken because sampling frames, matrices, analytical methods, detection limits and reporting thresholds differed substantially. Descriptively, several African and Asian surveys reported frequent AFB1 exceedances, whereas European and Chinese studies more often used liquid chromatography-based multi-analyte platforms that revealed broader co-occurrence profiles. These patterns should be interpreted as methodological and descriptive contrasts rather than proof of statistically significant regional differences (Table 1).

Exposure interpretation

From a risk-assessment perspective, the reported feed concentrations imply narrow safety margins for poultry. Assuming a daily feed intake of approximately 100 g/kg body weight, feed containing 20 µg/kg AFB1 corresponds to an intake of about 2 µg/kg body weight/day, while 100–200 µg/kg feed corresponds to 10–20 µg/kg body weight/day. Such exposures overlap with experimental doses associated with altered serum enzymes, histopathology, immunity and intestinal-barrier markers and co-exposure to DON, ZEA, OTA or fumonisins may further modify the response (25, 31–40).

Hazard characterisation and mechanistic insights for monitoring and risk interpretation

AFB1 is the most toxic and extensively characterised aflatoxin in poultry systems. After absorption, hepatic cytochrome P450 enzymes, particularly CYP1A5, convert AFB1 to the reactive AFB1-8,9-epoxide (AFBO). AFBO forms adduct with DNA and proteins, generates oxidative stress and initiates hepatocellular injury, reflected by increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and characteristic hepatic lesions (31–33). Fig. 2 summarises the pathway from contaminated feed to poultry toxicity and downstream consumer exposure, while Table 2

Table 1. Global prevalence of aflatoxins in poultry feeds (2020–25)

Region	Country/study	Matrix	Analytical method*	Aflatoxin range (µg/kg)	Sample size (n)	Co-occurrence	References
Africa	Uganda (Nakavuma 2020)	Maize bran, silverfish	VICAM AflaTest immunoaffinity column + fluorometric detection	>20 (all samples)	40 feeds (processors) + 27 feeds (farmers) + 20 ingredients	-	(6)
Africa	Ghana (Aboagye-Nuamah 2021)	Poultry feed	AgraStrip® total aflatoxin and Fumonisin quantitative LFD (Romer Labs)	25–120	350 prepared feeds	Fumonisin	(14)
Africa	Nigeria (Salisu 2020)	Commercial feed	Fourier-transform infrared spectroscopy (FTIR) after methanol-water extraction	12–200	15 feed samples	Fungal isolates	(24)
Africa	Senegal (Minougou 2023)	Poultry feed	HPLC (ISO 14718 method)	40–180	68 feed samples	-	(15)
Asia	Pakistan (Naveed 2022)	Poultry feed	Direct competitive ELISA (Neogen Veratox kit)	10–140	40 feed samples	-	(16)
Asia	India (Tamilmani 2021)	Experimental diets	TLC (thin-layer chromatography) quantification of dietary AFB1 and OTA	25–100	5 diet treatments; 240 broilers	OTA	(25)
Asia	China (Zhao 2021)	Feed nationwide	HPLC with fluorescence detection (LC-FLD)	5–85	3,507 total feed samples (2,090 ingredients; 1,417 complete feeds incl. 572 poultry feeds)	DON, ZEA	(7)
MENA	Jordan (Omar 2021)	Eggs (fried)	Lateral flow device (LFD) screening + LC-MS/MS confirmation	2–35	250 fried egg samples	OTA, DON, ZEA	(12)
MENA	Saudi Arabia (Gherbawy 2025)	Compound feed	HPLC for multiple mycotoxins	12–90	100 compound feed samples	Fungi	(19)
Europe	Spain (Muñoz-Solano 2023)	Feed samples	HPLC-FLD for AFs/OTA/ZEA + ELISA for DON/STER	5–40	400 compound feeds (including 100 poultry feeds)	OTA, DON, ZEA	(18)

VICAM: vision inspection camera; LFD: lateral flow device; HPLC: high-performance liquid chromatography; ELISA: enzyme-linked immunosorbent assays; LC-FLD: liquid chromatography with fluorescence detection; LC-MS/MS: liquid chromatography-tandem mass spectrometry; ZEA: Zearalenone; DON/STER: Deoxynivalenol/Sterigmatocystin.

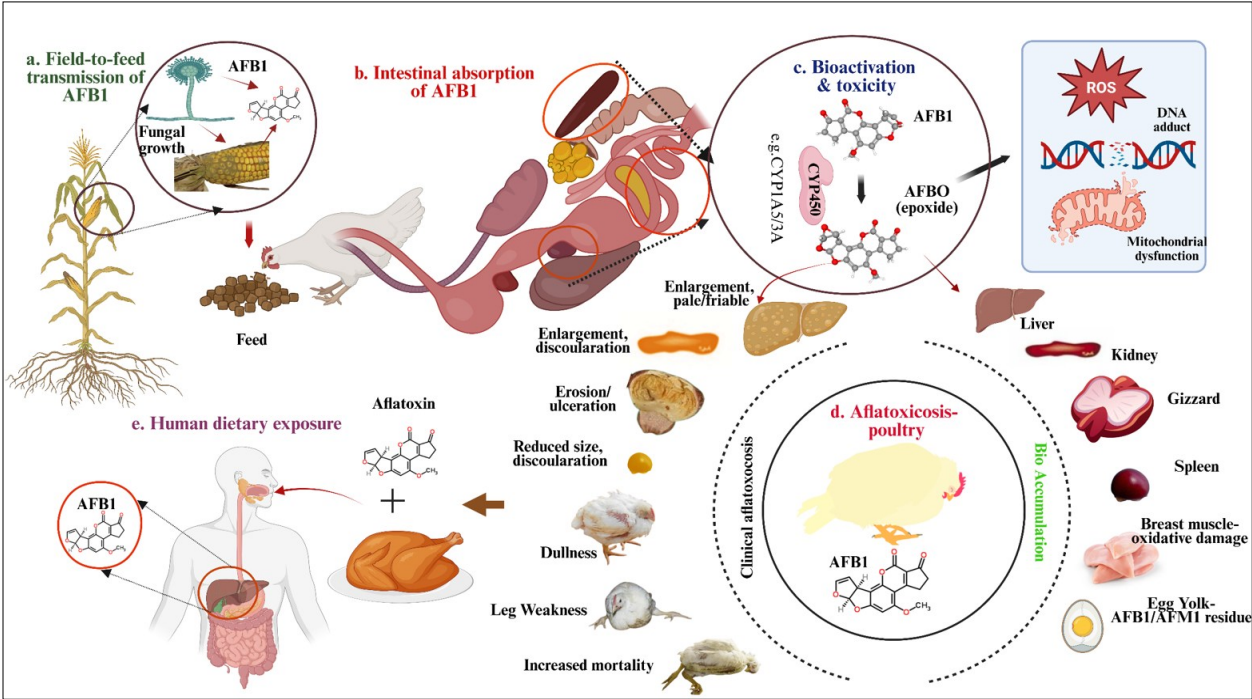


Fig. 2. Schematic pathway of aflatoxin B1 (AFB1) from crop to poultry and humans: (a) Field-to-feed transmission through contaminated maize or groundnut ingredients; (b) intestinal absorption; (c) CYP1A5-mediated bioactivation to AFBO, oxidative injury, DNA-adduct formation and mitochondrial dysfunction; (d) clinical and organ-level effects in poultry; and (e) human dietary exposure through contaminated poultry products. (Created in BioRender. C, J. (2026) <https://BioRender.com/tz8s2f1>).

lists representative target systems and biomarkers.

Oxidative stress and mitochondrial dysfunction are recurrent mechanisms. Studies report increased malondialdehyde (MDA), altered superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase activities, mitochondrial injury and DNA damage, which contribute to impaired growth and organ function (33, 34). Because these endpoints change across a range of exposure conditions, they are frequently used as early-response or surrogate biomarkers when mitigation strategies are evaluated.

Interactions among co-occurring mycotoxins require endpoint-specific interpretation. AFB1 and OTA converge on oxidative injury, mitochondrial dysfunction, impaired protein synthesis and hepatic or renal damage; AFB1 and fumonisins can jointly affect hepatic biotransformation, sphingolipid metabolism, intestinal integrity and immune competence and DON or ZEA may add epithelial, inflammatory and endocrine effects. Experimental studies report additive or synergistic effects for selected performance, biochemical, transcriptomic and histopathological outcomes, but antagonistic or non-additive responses can also occur when doses, timing and metabolism differ (25, 37–40). Consequently, co-occurrence should not be described as universally

synergistic and future studies should use field-relevant mixtures and report complete toxin profiles.

Beyond the liver, aflatoxins can suppress antibody responses, alter cytokine profiles and leukocyte indices, shorten intestinal villi, modify crypt architecture and reduce tight-junction proteins, thereby linking exposure with poorer nutrient use and increased disease susceptibility (35, 36). These effects also explain why probiotics, synbiotics and yeast derivatives can provide benefits even when complete toxin adsorption is not achieved.

Residue formation is central to consumer protection. AFB1 can be hydroxylated to AFM1 and other metabolites and residues have been detected in eggs, liver, kidney and muscle in surveys and controlled studies (11–13, 46, 47). Although tissue concentrations are usually lower than feed concentrations, repeated consumption and co-exposure justify risk-based monitoring of high-risk ingredients, eggs and offal.

Mitigation strategies for aflatoxicosis in poultry

Mitigation studies were synthesised by evidence strength rather than described study by study. Across the reviewed evidence and existing meta-analyses, clay-based binders and yeast-derived products showed the most consistent protection against AFB1,

Table 2. Organ systems, mechanisms of AFB1 toxicity and key biomarkers in poultry (2020–25)

Target system	Mechanism of toxicity	Biomarkers observed	Example study	References
Liver	CYP1A5 bioactivation, DNA adducts, necrosis	↑ALT, ↑AST, histopath lesions	Saleemi 2020; Yuan 2024	(33, 41)
Kidney	ROS generation, apoptosis, mitochondrial disruption	↑MDA, apoptosis markers	Damiano 2022; Tao 2021	(32, 42)
Immune	Cytokine suppression, impaired antibody titers	↓IL-2, ↓IFN-γ, ↓NDV titers	Hernández-Ramírez 2020; Jamil 2025	(35, 43)
Intestine	Tight-junction disruption, villus atrophy	↓Occludin, ↓Claudin	Choi 2020; Abd El-Hack 2023	(38, 44)
Bone	Reduced mineralisation	↓Tibial ash, ↓bone strength	Paneru 2024	(45)
Eggs/Residues	Metabolism to AFM1, deposition in eggs	AFM1 in yolk and albumen	Omar 2021; Alaboudi 2022	(12, 13)

CYP: cytochrome; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ROS: reactive oxygen species; MDA: malondialdehyde; IL: interleukin; IFN-γ: interferon-gamma; NDV: newcastle disease virus; AFM: aflatoxin M.

while probiotics, synbiotics, enzymatic detoxifiers and phytochemicals provided complementary benefits. No single additive was universally effective under all contamination patterns and performance depended on toxin profile, dose, formulation, inclusion rate and outcome measured (48–50). Representative mechanisms, outcomes and limitations are summarised in Table 3.

Clay minerals such as bentonite, zeolite, clinoptilolite and HSCAS reduce gastrointestinal absorption of AFB1 through adsorption and have repeatedly improved growth, feed conversion, serum biochemistry and hepatic or intestinal outcomes in broilers and layers (27, 51, 52, 75, 76). Yeast-cell-wall products provide beta-glucans and mannan-oligosaccharides that bind selected toxins and modulate immunity and the intestinal microbiota. An additive based on *Saccharomyces cerevisiae* RC016 reduced liver AFB1 residues and improved villus height in broilers chronically exposed to AFB1 (58) and red yeast and other yeast-derived products have also shown protective effects (48, 59).

These high-confidence tools nevertheless require product-specific appraisal. Non-selective adsorption by some clays may reduce the availability of vitamins, trace minerals, veterinary medicines or other feed additives, especially at high inclusion rates. Clay purity, mineral composition and binding specificity vary, while yeast-cell-wall composition and efficacy differ with strain and processing. Long-term evidence on nutrient utilisation, tissue residues and chronic safety remains limited for several commercial formulations. Economic feasibility is context-dependent and should account for additive cost, contamination prevalence, productivity recovery, testing expenses and avoided product rejection; products should therefore be selected using validated binding data and local cost-benefit analysis rather than treated as interchangeable universal solutions.

Probiotics and synbiotics provide a complementary biological layer through cell-wall binding, enzymatic transformation, microbiota modulation and barrier or immune support. Controlled studies with *Lactobacillus plantarum*, *Bacillus licheniformis* and combined prebiotic-probiotic formulations reported improvements in hepatic, intestinal, immune and performance outcomes, although responses were strain- and formulation-specific and viability during pelleting and storage remains a practical constraint (43, 44, 53, 55–57). Enzymatic detoxifiers can directly modify toxin structures, but their field evidence remains less mature; thermal stability, substrate specificity, transformation-product safety, cost and regulatory approval require further validation (60–62).

Phytochemicals such as curcumin, lycopene, berberine, silymarin, *Spirulina* and *Moringa oleifera* Lam. primarily act through antioxidant, anti-inflammatory and immunomodulatory pathways and can reduce biochemical or histological injury in controlled trials (32, 63–67, 74, 77, 78). Their reproducibility is limited by variation in plant source, extraction, bioavailability and active-compound concentration, so standardised formulations and direct comparisons with established binders are needed.

Nanoparticles leverage increased surface area and engineered surface chemistry to enhance toxin binding and antifungal activity. Nanosilica and chitosan-based materials have shown experimental efficacy, while metallic nanoparticles can inhibit *Aspergillus* growth or aflatoxin biosynthesis (68–70). However, translation requires evidence on oral toxicity, tissue retention, worker exposure, environmental release, nutrient interactions, batch consistency and regulatory classification. Plasma-activated water (PAW) has been reported to reduce AFB1, OTA and fumonisins

in poultry-feed matrices under experimental conditions, but treatment uniformity, reactive-species stability, energy and water requirements, nutrient oxidation, throughput, detoxification-product safety and regulatory acceptance remain unresolved (29). Postbiotics offer improved processing stability, but evidence is still limited and product definitions require standardisation (71).

Multi-agent programmes that combine adsorption with biotransformation, antioxidant support and gut or immune modulation are biologically plausible and often outperform single-component approaches under multi-mycotoxin exposure (49, 50, 73, 74). Nevertheless, increasing formulation complexity can raise cost and complicate quality control. The most defensible strategy is therefore to match validated interventions to locally measured toxin profiles and to integrate them with upstream crop management, drying, storage, segregation and risk-based monitoring.

Detection and monitoring

Reliable detection underpins surveillance, enforcement and evaluation of mitigation. Thin-layer chromatography (TLC) remains useful for low-cost screening of gross contamination, while high-performance liquid chromatography with fluorescence detection (HPLC-FLD) provides sensitive confirmatory analysis for a limited number of aflatoxins. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) and ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) enable simultaneous multi-mycotoxin quantification and are the preferred reference-laboratory platforms, but require substantial capital investment, skilled personnel and rigorous quality assurance (4, 79–81). Table 4 compares the principal methods.

Enzyme-linked immunosorbent assays (ELISAs), lateral-flow devices (LFDs) and quantitative immunochromatographic strips provide rapid, relatively inexpensive screening at farms and feed mills. Their limitations include matrix effects, cross-reactivity, lot-to-lot variability and restricted quantitative ranges; positive, borderline or enforcement-relevant results should therefore be confirmed chromatographically (6, 12, 14–16, 21, 23, 46). Biosensors and smartphone-linked platforms offer portability and low analytical limits under controlled conditions, but most remain at proof-of-concept or early validation stages and require inter-laboratory and feed-matrix validation (79, 82–84).

A tiered testing cascade can balance cost and analytical certainty (Fig. 3). Tier 1 uses LFDs or simple ELISA for high-frequency screening; Tier 2 uses quantitative immunoassays for management decisions; and Tier 3 applies LC-MS/MS or UHPLC-MS/MS to selected or critical lots for definitive multi-mycotoxin profiling. Embedding this cascade within risk-based sampling allows limited confirmatory capacity to be directed toward high-risk ingredients, seasons, suppliers and borderline lots (4, 7, 80, 85, 86).

Public-health and One Health implications

Residues and consumer exposure

Carry-over of AFB1 and its metabolites into eggs and edible tissues provides a direct route of human exposure. Surveys from Jordan and other settings detected AFB1, AFM1 and co-occurring mycotoxins in eggs, liver, kidney and muscle, with liver and kidney generally showing higher concentrations than muscle (12, 13, 46, 47). Cooking reduces some residues only partially and formal risk assessments indicate that frequent consumption of contaminated eggs or offal can contribute materially to exposure in high-burden settings. However, heterogeneity in sampling, analytical methods

Table 3. Comparative summary of mitigation strategies against aflatoxins in poultry (2020–25)

Strategy	Examples/agents	Primary mechanism	Evidence strength	Key outcomes	Main limitations	References
Clay-based binders	Bentonite, zeolite, HSCAS, smectite-based products	Physical adsorption of AFB1 in the gut; reduced systemic bioavailability	High for AFB1 (multiple <i>in vivo</i> broiler and layer trials)	Reduced liver lesions, lower ALT/AST, improved FCR and weight gain, partial reduction of tissue residues	Toxin-specific (less effective for DON, fumonisins); potential binding of vitamins/minerals; efficacy declines at very high contamination levels	(27, 51, 52)
Other adsorbents	Poultry-litter biochar, humic acids	High surface area adsorption; cation exchange; possible antioxidant effects	Moderate (<i>in vivo</i> studies + <i>in vitro</i> binding data)	Reduced liver damage, improved haematology and immune indices	Limited multi-mycotoxin data; variability in source materials and production conditions	(53, 54)
Biological agents-probiotics	<i>Lactobacillus plantarum</i> , <i>Bacillus licheniformis</i> , indigenous strains	Cell-wall binding of aflatoxins; enzymatic biotransformation; modulation of gut microbiota and immunity	Moderate-high (multiple trials, some co-infection models)	Improved liver histology, reduced mortality, better gut integrity, protection in necrotic enteritis and Salmonella co-challenges	Strain-specific effects; viability during feed processing and storage; limited data on residue reduction	(43, 55, 56)
Biological agents-prebiotics & synbiotics	MOS, FOS, inulin + probiotic blends	Support growth of beneficial microbiota; indirect toxin degradation; improved barrier function and immune status	Moderate (<i>in vivo</i> studies, some reviews/meta-analyses)	Improved villus height: crypt ratio, higher vaccine titers, lower oxidative stress	Typically, weaker direct binding than clays; formulation-dependent; cost considerations	(44, 48, 53, 57)
Yeast and yeast derivatives	<i>S. cerevisiae</i> cell walls, β -glucans, MOS, red yeast (<i>Sporidiobolus pararoseus</i>)	Adsorption via β -glucans and MOS; immune stimulation; microbiota modulation	High (substantial trial base + reviews)	Reduced AFB1 residues in liver, improved intestinal morphology, better performance and vaccine responses	Quality varies between commercial products; may need combination with clays or enzymes in high-risk settings	(48, 58, 59)
Enzymatic detoxifiers	Engineered oxidoreductases, esterase blends, commercial detoxifier mixes	Structural degradation of AFB1 (e.g., lactone ring opening) to less toxic metabolites	Emerging moderate (promising <i>in vivo</i> and <i>in vitro</i> , still limited field-scale data)	Improved growth, reduced serum liver enzymes and oxidative stress markers	Cost; thermal stability in pelleting; regulatory approval; specificity for multiple co-occurring mycotoxins	(60–62)
Phytochemicals & nutraceuticals	Curcumin, nano-curcumin, lycopene, berberine, <i>Moringa oleifera</i> leaves, silymarin, Spirulina	Antioxidant, hepatoprotective, anti-inflammatory, immunomodulatory	Moderate (multiple experimental trials, some combined toxin models)	Lower MDA and other oxidative markers, improved liver and kidney histology, improved performance	Variability in plant sources and active compound content; need for standardized formulations and dose-response data	(32, 63–67)
Nanotechnology-based adsorbents	Nano silica, chitosan nanoparticles, metal nanoparticles activity; ROS modulation (Ag, ZnO)	Enhanced surface binding; antifungal activity; ROS modulation	Emerging (mostly experimental/short-term <i>in vivo</i> + <i>in vitro</i>)	Improved performance and liver histology; inhibition of <i>Aspergillus</i> growth and aflatoxin biosynthesis	Safety and residue concerns; regulatory acceptance; scaling and cost	(68–70)
Novel tools	Plasma-activated water, postbiotic yeast blends, essential oil/organic acid combinations	<i>In situ</i> degradation of toxins; stable microbial metabolites; antifungal and anti-inflammatory effects	Emerging (limited but promising studies)	Reduction in AFB1, OTA and fumonisins in feed; better gut morphology and immune markers	Technology access; optimization for commercial feed mills; need for long-term field validation	(29, 71, 72)
Multi-agent formulations	Combinations of clays, yeast derivatives, probiotics, enzymes, phytochemicals	Multi-modal: adsorption + biotransformation + antioxidant and immune support	Growing (reviews and meta-analyses show superior performance vs single agents)	Consistently improved growth, FCR, organ health and in some cases lower residues under multi-mycotoxin challenge	Formulation complexity; cost; need to tailor combinations to regional mycotoxin patterns	(49, 50, 73, 74)

ALT: alanine aminotransferase; AST: aspartate aminotransferase; DON: Deoxynivalenol; HSCAS: hydrated sodium calcium aluminosilicate; AFB: Aflatoxin B; FCR: feed conversion ratio; MOS: Mannan oligosaccharides; FOS: Fructooligosaccharides; MDA: malondialdehyde; ROS: reactive oxygen species; OTA: Ochratoxin A.

Table 4. Comparison of detection and monitoring methods for aflatoxins in poultry feed and products

Method	Typical use	Relative cost	Relative sensitivity / specificity	Typical LOD/ LOQ range for AFB1 in feeds (µg/kg) *	Main strengths	Main weaknesses	Example use	References
TLC	Basic screening in small labs; confirmation for high-level contamination	Low	Low-moderate	LOD ~2-10; LOQ ~5-20	Very cheap; minimal instrumentation; useful where resources are limited	Labor-intensive; lower sensitivity and poor multi-toxin coverage; semi-quantitative	Salisu 2021	(81)
HPLC (often FLD)	Regulatory and research labs for single or few mycotoxins	Moderate-high	High	LOD ~0.1-1; LOQ ~0.3-3	Robust quantification; widely standardised; suitable for confirmatory analysis	Requires derivatisation for some aflatoxins; slower throughput than LC-MS/MS; higher operating costs than ELISA	Khorrami 2022	(17)
ELISA	High-throughput screening of feeds in mills and regional labs	Low-moderate	Moderate-high (for target analyte)	LOD ~0.5-2; LOQ ~1-5	Rapid (dozens of samples per run); relatively cheap; easy to perform	Antibody cross reactivity and matrix effects; usually single-toxin; positive results require confirmation	Naveed 2022; Kabeta 2024	(16, 20)
LC-MS/MS	Gold-standard multi-mycotoxin analysis in reference labs	High	Very high	LOD ~0.01-0.1; LOQ ~0.03-0.3	Simultaneous detection of multiple mycotoxins with excellent sensitivity and specificity; sub-µg/kg limits	High capital and running costs; technical expertise required; not widely available in small labs	Zhao 2021; Sarwat 2022	(7, 80)
UHPLC-MS/MS	High throughput national surveillance programs	High	Very high	LOD ~0.01-0.1; LOQ ~0.03-0.3	Faster separations and lower solvent use vs conventional LC-MS/MS; ideal for large monitoring datasets	Same limitations as LC-MS/MS regarding cost, maintenance and expertise	Muñoz-Solano 2023	(18)
GC-MS	Research on degradation products and metabolite identification	High	Very high	LOD ~0.1-0.5; LOQ ~0.3-1	Structural confirmation of degradation products; useful for mechanistic detoxification studies	Requires derivatisation; complex sample preparation; not practical for routine feed testing	Elkenany 2021	(79)
Biosensors (electrochemical/optical)	On-site quantitative screening in feed mills or warehouses	Moderate (device) + low (per test)	High (when validated)	LOD commonly ~0.05-1; LOQ ~0.1-2	Portable; rapid; potential for multi-analyte detection; can integrate with smartphone platforms	Field validation still limited; devices not yet widely commercialised in all regions	Elkenany 2021	(79)
Lateral flow devices (LFDs)	On-farm or mill-side qualitative/semi-quantitative screening	Very low-low	Moderate	Visual threshold typically 2-20 (kit-dependent)	Simple yes/no or threshold-based results within minutes; minimal training required	Semi-quantitative at best; can yield false positives/negatives; best used as first-line screen	Omar 2021	(12)
Quantitative immunochromatographic strips	Intermediate confirmation before LC-MS/MS	Low-moderate	High	LOD ~0.5-2; LOQ ~1-5	Multiplex capability; portable readers provide numerical values; faster than chromatography	More expensive than basic LFDs; require calibration and maintenance of readers	Khalifa 2022	(14)
Omics-based biomarkers (transcriptomic/metabolomic)	Research on early aflatoxicosis in poultry	High (research only)	Potentially very high	Not directly expressed as µg/kg (indirect exposure markers)	Detect early, subclinical tissue stress; provide mechanistic insight; candidate tools for One Health surveillance	Currently experimental; not standardised for routine use; high data-analysis demands	Choi 2020; Tao 2021	(38, 42)

HPLC: high-performance liquid chromatography LOD: limit of detection; LOQ: limit of quantification; LC-MS/MS: liquid chromatography-tandem mass spectrometry LFD: lateral flow device; GC-MS: gas chromatography-mass spectrometry; UHPLC-MS/MS: ultra-high-performance liquid chromatography-tandem mass spectrometry; ELISA: high-performance liquid chromatography TLC: thin layer chromatography.

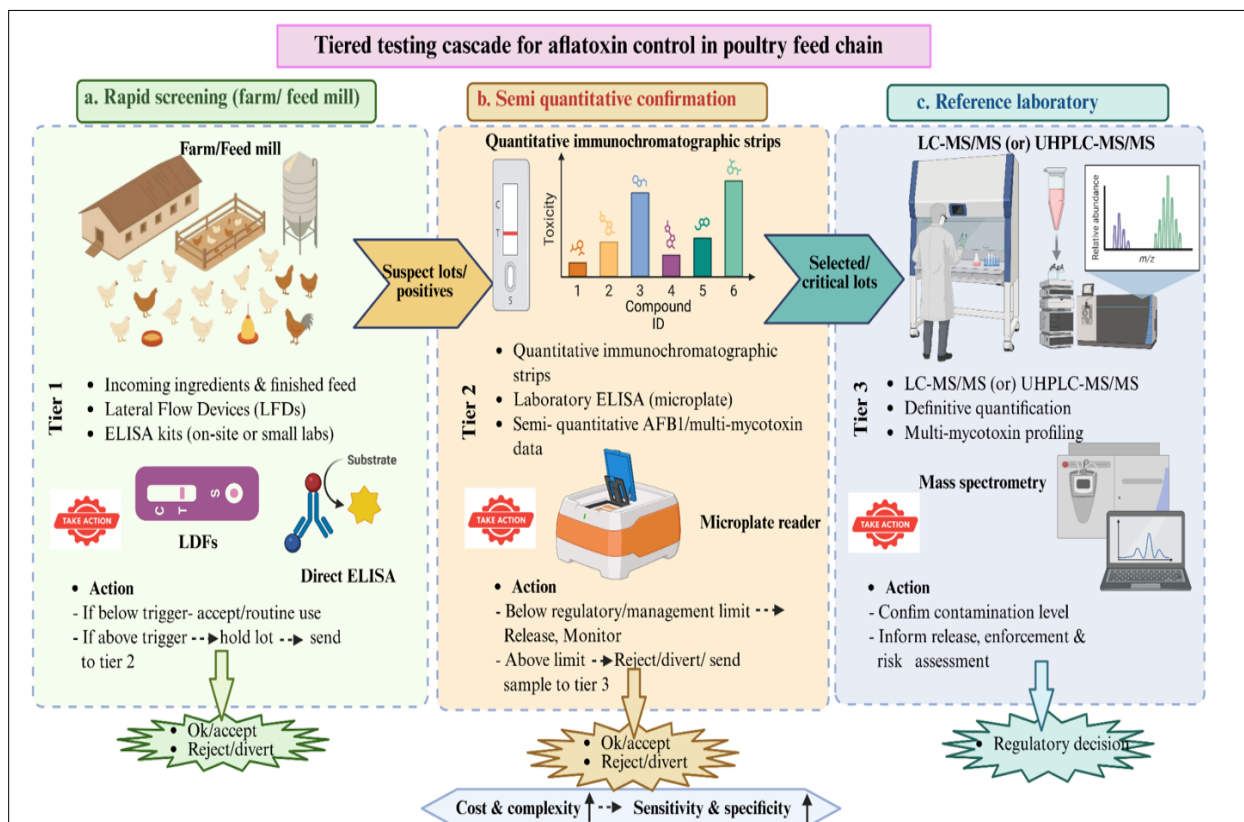


Fig. 3. Tiered testing cascade for aflatoxin control in poultry-feed chains: Tier 1 uses lateral-flow devices or simple enzyme-linked immunosorbent assays for rapid screening; Tier 2 uses quantitative immunoassays for management decisions; and Tier 3 uses LC-MS/MS or UHPLC-MS/MS for definitive multi-mycotoxin quantification and regulatory action. (Created in BioRender. C, J. (2026) <https://BioRender.com/tz8s2f1>).

and consumption assumptions prevents a pooled cross-regional risk estimate.

A One Health lens

Aflatoxins in poultry systems exemplify a One Health problem linking crops, feed, animal health, food products and human exposure. The same AFB1 that impairs growth, immunity and vaccine responsiveness in broilers and layers can contaminate eggs and tissues destined for consumption, while its formation is driven by crop-fungus interactions shaped by climate, agronomy, drying and storage (2, 8, 86, 87). Immunosuppression may also increase susceptibility to enteric and respiratory pathogens and indirectly increase antimicrobial use (36, 37). Upstream interventions-including resistant crops, atoxigenic biocontrol, rapid drying, improved storage and feed-mill quality control-therefore provide simultaneous benefits for poultry productivity, residue reduction and public health (88–90).

Policy and surveillance

Effective control requires harmonised standards, transparent reporting and linked screening-confirmation networks. Many small feed mills and informal markets operate with limited laboratory access and inconsistent enforcement, so surveillance should prioritise high-risk commodities, seasons and suppliers and should include multi-mycotoxin coverage where feasible (7, 20–22, 80, 86, 91). Eggs and offal may also serve as sentinel matrices for upstream control failures.

Socio-economic implications and equity

Subclinical aflatoxicosis can substantially increase feed cost per unit of meat or egg through poorer growth, feed conversion and flock uniformity, whereas severe contamination can cause product

rejection and reputational loss (8, 10, 26, 50). These effects disproportionately burden small and medium producers with limited access to reliable feed, testing and validated additives. Control programmes should therefore evaluate affordability and distributional effects so that stricter food-safety enforcement does not unintentionally reduce access to poultry protein in low-income settings. Major One Health and socio-economic dimensions of aflatoxin contamination in poultry systems are synthesised in Supplementary Table 3.

Conclusion and future perspectives

This systematic review synthesised 78 peer-reviewed primary studies published between 2020 and 2025. AFB1 remained the dominant aflatoxin in poultry-feed chains and frequently co-occurred with deoxynivalenol (DON), zearalenone (ZEA), ochratoxin A (OTA) and fumonisins. Several African and Asian surveys reported frequent exceedances, while multi-analyte studies from China and Europe demonstrated the breadth of co-contamination; direct regional ranking was not possible because surveillance designs and analytical methods were heterogeneous.

Mechanistic evidence converged on cytochrome P450-mediated bioactivation, oxidative and mitochondrial injury, immune dysfunction and intestinal-barrier disruption. Mixture effects were endpoint- and dose-dependent rather than universally synergistic. Clay and yeast-derived products had the most consistent mitigation evidence, but product specificity, nutrient interactions, long-term safety and economics require critical appraisal. Probiotics, synbiotics, enzymes and phytochemicals offer complementary functions, while nanomaterials, plasma-activated water (PAW) and

postbiotics require stronger evidence on safety, scale-up and regulation.

Rapid immunoassays are practical for routine screening, whereas liquid chromatography-tandem mass spectrometry (LC-MS/MS) remains essential for confirmatory multi-mycotoxin analysis. Future priorities include field-relevant mixture studies, harmonised reporting, commercial-scale validation of mitigation tools, stronger residue evidence and surveillance capacity in high-burden feed chains. Collectively, these efforts are essential to reduce AFB1 exposure in poultry production systems, ensuring sustainable productivity while safeguarding animal and public health.

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Authors' contributions

MV designed the study. VN experimented and drafted the JC contributed to the original draft preparation, methodology, formal analysis and visualisation. PV contributed to supervision and writing the review and editing. BD contributed to software analysis and conceptualisation. PV, SS and TM contributed to the review and editing of the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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Declaration of generative AI and AI-assisted technologies in the writing process

During revision of this manuscript, the authors used OpenAI ChatGPT to assist with language editing, readability, formatting consistency and organisation of responses to reviewers. The tool was not used to select studies, extract or generate data, perform statistical analyses, verify references or formulate scientific conclusions. All suggested text was critically reviewed, corrected and approved by the authors, who take full responsibility for the accuracy and integrity of the manuscript.

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