

Occurrence of major mycotoxins in non-corn forages for dairy cattle: A survey of silages and hays from Italian farms

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Abstract: The occurrence of mycotoxins in ruminant feeds has been traditionally linked to corn silage, but alternative forages such as cereal straw, grass silages, sorghum, and legume hays may also contribute to exposure. We investigated the main regulated mycotoxins in 977 forage samples from Italian farms, including 642 silages (wheat, grasses, sorghum) and 335 hays (wheat straw, grasses, alfalfa). Samples were analyzed for deoxynivalenol (**DON**), zearalenone (**ZEN**), and total fumonisins (**FUM**) using accredited methods. Non-corn forages were not exempt from contamination, showing variable prevalence and concentrations depending on forage type and botanical origin. DON was the most prevalent, ZEN showed moderate prevalence with rising concentrations in recent years, and FUM occurred less frequently but with occasional and not significant high levels. These findings highlight the need for continuous monitoring of all forage types, suggesting that mycotoxin risk assessment in dairy diets should extend beyond corn silage.

Mycotoxin contamination represents a well-documented issue in animal production, with significant implications for both livestock health and food safety (Akinmoladun et al., 2025). Mycotoxins are toxic secondary metabolites produced mainly by fungi belonging to the genera *Aspergillus*, *Fusarium*, and *Penicillium* (Gallo et al., 2015). Their occurrence in forages used for ruminant feeding can lead to a wide range of consequences, from subclinical effects such as reduced feed intake, impaired feed efficiency, and immune or reproductive disturbances, to more severe outcomes under chronic exposure (Xu et al., 2022; Yang et al., 2020). Scientific literature and official monitoring programs have strongly focused on corn and whole-plant corn silage, which are recognized as highly susceptible substrates for fungal colonization (Ogunade et al., 2018; Ghilardelli et al., 2022). However, dairy cattle diets also rely extensively on alternative forages, including small-grain cereal silages, sorghum, grasses, and hay or straw from cereals and legumes such as alfalfa (Burke et al., 2007; Cattani et al., 2017; Xia et al., 2025). Despite their widespread use across Italian and European farms, knowledge on mycotoxins contamination about these non-corn forages remains limited and fragmented. Previous reports have highlighted the presence of *Fusarium*-derived toxins, such as deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FUM), and T-2/HT-2 toxins, in cereal straws and minor grains, as well as aflatoxins and ochratoxin A in hays subjected to inadequate storage conditions (Gozzi et al., 2024; Yu & Pedroso, 2023). Contamination levels can be influenced by several factors, including forage species, harvesting stage, climatic conditions during drying or ensiling, and conservation practices at the farm level (Manni et al., 2022). The present study aims to systematically investigate the occurrence of major regulated mycotoxins in non-corn forages,

analysing a large dataset of 977 samples collected from dairy farms located in Northern Italy, mainly in the forage-producing lowland areas of Piedmont, Lombardy, Veneto, and Emilia-Romagna. The temporal distribution was not fixed across years; sample submissions occurred throughout the year, with variable proportions in each quadrimester depending on forage availability and routine monitoring practices. Across 2018-2025, 28-66% of samples were delivered in January-April, 18-37% in May-August, and 28-51% in September-December. Climatic conditions of the mentioned forage-producing areas were summarized for the investigation (ISTAT, 2024; SNPA, 2025). Annual mean temperatures ranged between 14.2 and 15.3 °C, with minimum and maximum means spanning 9.4-10.5 °C and 18.6-19.9 °C, respectively. Relative humidity varied from 71.7% to 78.5%, and cumulative precipitation from 528 to 1,104 mm. Specifically, 2018-2019 were warm and humid years with high rainfall (815-844 mm), 2020-2022 were markedly drier (528-653 mm), 2023 showed moderate precipitation (717 mm), whereas 2024 recorded the highest rainfall of the series (1,104 mm). The dataset includes 642 silages (mainly wheat, grasses, and sorghum) and 335 hays (primarily wheat straw, grasses, and legumes such as alfalfa). The silages mainly comprised wheat (n = 202), mixed grasses and autumn cereals (n = 160), sorghum (n = 121), triticale (n = 42), alfalfa and soybean mixtures (n = 38), ryegrass (n = 38), barley (n = 33), rye (n = 7), and oat (n = 1). The hays and straws included wheat straw (n = 124), grass mixtures (n = 102), grass-legume mixtures (n = 33), alfalfa hay (n = 30), wheat hay (n = 20), ryegrass hay (n = 16), oat hay (n = 7), triticale hay (n = 1), barley hay (n = 1), and millet hay (n = 1). The category “mixed-cereal silages” included autumn-winter forage mixtures mainly composed by small-grain cereals

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The list of standard abbreviations for JDSC is available at [adsa.org/jdsc-abbreviations-25](https://www.adsa.org/jdsc-abbreviations-25). Nonstandard abbreviations are available in the Notes.

(e.g., wheat, triticale, barley, and ryegrass). Forage sampling followed the Laboratorio di Analisi Zootecniche (Mancinelli Dr. Eugenio & Dr. Marco S.a.s., <https://www.lazoovet.it/>, Gonzaga, Italy) internal procedure A1-IO-ACC rev.2 and the principles of EU Regulation 152/2009. Silage samples were collected using a multi-point random approach from at least five positions along the silage face or from multiple cores in wrapped bales; hay samples were taken with a mechanical corer from different bales and depths to avoid leaf-stem segregation. Subsamples were combined into a single composite sample per lot, with a minimum representative quantity of approximately 1.5 kg for silages and 0.5-1 kg for hays. For mycotoxin determination, larger composite masses were occasionally prepared to improve representativeness before milling. Samples were sealed in robust polyethylene bags (non-biodegradable), kept refrigerated (4-10 °C) or frozen when necessary, transported in insulated boxes with cooling packs if delivery exceeded one day, and homogenized upon arrival at the laboratory for analysis. For statistical purposes, the results of mycotoxin analyses were considered collectively, combining silages and hays into a single dataset of 977 samples. Mycotoxin concentrations were expressed on a DM basis. From 2018 to 2025, ZEN, DON, and total FUM were determined by competitive ELISA (RIDASCREEN® and Tecna/Eurofins) at the Laboratorio di Analisi Zootecniche. All forage samples were numbered, labelled, and milled to 0.5 mm. Silages were first homogenized with a blade cutter, then dried at 65 °C for ~16 h before milling; hay moisture was measured with a thermobalance. For ZEN and FUM, 5.00 ± 0.05 g of sample were extracted with methanol-water (70:30, v/v) plus NaCl; for DON, 5.00 g were extracted with 50 mL of distilled water. Extracts were clarified by sedimentation, filtration through Whatman No. 1 paper, and pH adjustment (6.5-7.5). SPE purification was applied to ZEN (Romer MycoSep® AlfaZon) and DON (Romer MycoSep® Trich), while FUM was analyzed without SPE. Purified extracts were stored at +4 °C for short-term analysis or -15 °C for up to five days. ELISA plates pre-coated with specific antibodies were processed according to kit protocols, including incubation with HRPO conjugates, washing, TMB development, and acid stopping. Optical density at 450 nm was used to construct %B/B₀ standard curves (where B represents the absorbance of the standard or sample and B₀ that of the zero standard) and interpolate sample concentrations via cubic spline regression (RidaSoft), adjusting for sample weight and dilution. Each analytical batch included duplicate QC materials and was monitored with control charts; the method was externally validated through participation in the 2023 BIPEA 31E mycotoxin proficiency test on forage matrices. All statistical analyses were performed using R (version 4.4.1; R Foundation for Statistical Computing, Vienna, Austria) within the RStudio environment. To assess potential temporal trends in mycotoxin contamination, we applied weighted linear regression (WLR) models, where annual mean concentrations of each mycotoxin (DON, ZEN, and total FUM) were regressed against sampling year. The number of samples analyzed per year showing quantitative values > limit of quantification (LOQ) was used as a weighting factor in order to account for the unequal sampling effort across years. Model fit and statistical significance of regression coefficients were evaluated using standard diagnostics (t-tests for coefficients, F-test for overall model fit, and R² values for variance explained). Graphical visualization of the data was performed using the ggplot2 package, including scatterplots of annual mean

concentrations scaled by sample size and regression lines with 95% confidence intervals. A total of 977 forage samples (silages and hays) were analyzed over a 7-year period for the presence of ZEN, DON, and total FUM. Table 1 summarizes the number of samples analyzed per forage type, the proportion of positive samples above the LOQ, and the mean concentration of positive samples expressed in µg/kg DM. Overall, DON was the most frequently detected mycotoxin, while FUM showed lower prevalence across all forage types. Variations in occurrence and concentration were observed depending on the botanical composition and type of forage. As a general consideration, DON was the most prevalent mycotoxin, detected in the 48% of the analyzed samples, with the highest concentrations observed in straw (average: 950 µg/kg DM) and mixed-cereal silages (average: 1358 µg/kg DM). ZEN showed a lower prevalence overall, but sorghum silages and straw had notably higher positive rates and concentrations (Table 1). Total FUM were less frequently detected, with moderate average concentration values; the highest occurrence was observed in sorghum silages, while legume hay showed minimal contamination. These results highlight that non-corn forages are sensitive to mycotoxin contamination, and both forage type and botanical composition can potentially affect the risk of exposure. The differential occurrence of DON, ZEN, and FUM across forage types can be partly explained by the ecological niche of their main producing fungi. *F. graminearum* and related species, responsible for DON and ZEN biosynthesis, are strongly associated with cereal residues and straw, which explains the high positivity in wheat straw and mixed-cereal silages (Gozzi et al., 2024). In contrast, FUM are typically produced by *F. verticillioides* and *F. proliferatum*, fungi better adapted to warm and dry conditions, which may account for their sporadic but sometimes severe contamination in sorghum silages (Del Palacio et al., 2016). Legume-based forages, by comparison, tend to exhibit lower contamination rates, likely due to differences in plant physiology and structural composition, which reduce susceptibility to *Fusarium* colonization (Munkvold et al., 2021). These species-specific interactions highlight how both botanical origin and ensiling dynamics shape the mycotoxin risk profile in non-corn forages. The annual analysis of 540 forage samples for ZEN (2018-2025) showed moderate variability in both prevalence and concentration over the years. Positivity rates fluctuated between 18% and 44%, with the highest proportion of positive samples observed in 2019-2020 (44%). Mean concentrations of positive samples ranged from 55 µg/kg in 2022-2023 to 193 µg/kg in 2024-2025, indicating a marked increase in the last year of observation (Figure 1). Notably, despite some fluctuations in prevalence, the upward trend in mean concentration in 2024-2025 suggests that ZEN contamination became more pronounced, particularly in certain forage types, potentially reflecting climatic or agronomic variations. DON was consistently the most prevalent mycotoxin across all annual periods, with positivity rates ranging from 42% to 62%. The 2018-2019 season showed an unusually high mean concentration of 4099 µg/kg, markedly higher than all subsequent years. After this peak, prevalence remained relatively stable (42-46%), while mean concentrations of positives decreased substantially to 423-698 µg/kg, with a gradual increase in the last two years (2023-2025). This pattern indicates that, while DON is widely distributed in non-corn forages, extreme contamination events may occur sporadically, likely influenced by environmental conditions or crop management. Total FUM were generally less

prevalent, with annual positivity rates ranging from 9% to 40%. Low sample numbers in the early years (2018-2020) limit trend interpretation. However, from 2020 onward, there was a notable increase in both prevalence and concentration, peaking in 2023-2024 with 40% positive samples at a mean concentration of 838 $\mu\text{g/kg}$. Interestingly, the 2024-2025 period shows a decline in prevalence (11%) but an increase in mean concentration of positives to 1439 $\mu\text{g/kg}$, suggesting that fewer samples were contaminated, but those affected exhibited higher toxin loads. This sporadic distribution is consistent with FUM occurrence in non-corn forages, which is generally lower than in corn-based feeds. To investigate potential temporal trends in silage contamination, we applied WLR models, where annual mean concentrations of each mycotoxin (from 2018-2019 up to 2024-2025) were regressed against sampling year. Weights were assigned based on the number of samples analyzed per year, in order to account for the unequal sample size across years and provide a more robust estimate of overall tendencies. The weighted regression curves for each mycotoxin are reported in Figure 2. The regression model indicated a negative slope for DON concentrations over the study period ($\beta = -262 \mu\text{g/kg per year}$), suggesting a possible decline. However, the association was not statistically significant ($P = 0.236$), and the explained variance remained scarce ($R^2 = 0.27$). These results imply that DON contamination did not follow a consistent linear trend across the investigated years. For ZEN, the model estimated a positive slope corresponding to an annual increase of approximately $+19 \mu\text{g/kg}$ ($\beta = 19.5 \mu\text{g/kg per year}$). The association approached statistical significance ($P = 0.073$), with the model explaining nearly half of the weighted variance ($R^2 = 0.51$). Although the overall regression was not statistically significant, the highest ZEN concentrations were observed in the last sampling years (i.e. 2024-2025), particularly in sorghum silages and straw, indicating that these forage types contributed most to the apparent upward pattern. By contrast, total FUM concentrations did not show any evidence of temporal variation. The regression slope was slightly positive ($+58 \mu\text{g/kg per year}$), but highly uncertain, and not statistically significant ($P = 0.701$). The explained variance was negligible, indicating that year-to-year changes in FUM levels were not captured by a linear trend model. Therefore, the weighted regression approach confirmed the complexity of interpreting temporal dynamics in mycotoxin contamination. While DON showed a numerical tendency to decline, the lack of statistical significance reflects the nature of *Fusarium* epidemics, which are strongly dependent on seasonal climatic factors such as rainfall during flowering and pre-harvest periods (Chandelier et al., 2011). Conversely, the near-significant upward trajectory of ZEN is noteworthy, as it suggests a progressive increase in contamination pressure that may mirror shifts in rainfall distribution and relative humidity during forage harvest, as also observed by Moraes et al. (2023). The absence of any consistent pattern for FUM further underlines that these toxins are likely driven by episodic, localized events rather than long-term regional trends. Such variability emphasizes the importance of adopting flexible monitoring strategies rather than assuming predictable trajectories. A limitation of this survey is the heterogeneous sampling across years and forage types, which may have affected trend estimates despite weighted regression. The focus on DON, ZEN, and total FUM excluded other relevant toxins like T-2/HT-2 or emerging *Fusarium* metabolites. Additionally, ELISA assays were used for rapid, cost-effective quantification,

offering high throughput and applicability to complex feeds, but are subject to cross-reactivity, matrix effects, and higher detection limits than more accurate LC-MS/MS. Thus, results should be seen as semi-quantitative, yet sufficient for monitoring trends and relative differences. Despite these limitations, the dataset of nearly 1,000 samples over seven years provides robust evidence that mycotoxin risk extends beyond corn silage. To summarize, this comprehensive survey revealed that ZEN was detected on average in approximately 25% of all forage samples. The mean concentration of ZEN was approximately twice as high in grasses compared to legumes, both in silages and hays (the 56% positivity observed in legume silages was not considered due to the very small sample size, $n = 18$). Within the grass group, straw exhibited the highest mycotoxin load. Over the years, ZEN was found in 30-40% of samples, with concentrations ranging from 60 to 110 $\mu\text{g/kg}$. The period from July 2024 to June 2025 showed the largest increase, with mean concentrations nearly doubling to 193 $\mu\text{g/kg}$. DON was present in approximately 50% of samples, with no major differences between forage types, except for wheat straw, which stood out both for the proportion of positive samples and the mean toxin concentration. Silages of mixed grasses also contained relatively high DON levels, although they were less widely distributed compared to straw. The 2018-2019 season was notable for extremely high DON concentrations, while in subsequent years prevalence remained stable and mean concentrations gradually increased. The unusually high DON concentrations observed in 2018-2019 coincided with years characterized by elevated humidity and precipitation across the main forage-producing regions (relative humidity 73-75%; precipitation 815-844 mm), whereas 2020-2022 were markedly drier (528-653 mm). Although annual climatic means cannot fully capture crop-specific exposure during critical phenological phases, the wetter conditions of 2018-2019 are consistent with environmental scenarios known to promote *F. graminearum* development and DON accumulation. Total FUM were detected in approximately 20% of all forages, with legume hays appearing largely free of contamination. The number of samples analyzed prior to early 2023 was too limited to assess temporal trends; however, from 2023 onward, FUM were more frequently detected, albeit at relatively low concentrations. From a practical standpoint, these findings have implications for dairy cattle feeding strategies and feed safety assessment. It is well known that *Fusarium*-produced mycotoxin contamination typically has a negative effect on cow's performance as reviewed by Gallo et al. (2022), even when the levels at which these effects can be detected in commercial farms are still questionable and largely discussed by the scientific community and dairy technicians. Several authors (Seeling et al., 2006; Jovaisiene et al., 2016; Hartinger et al., 2022) reported that contamination levels under the recommendation proposed by EFSA (2006) could have negative impact on milk production and quality, reproductive performances as well as animal health status. Among these, *in vivo* trials investigating effect of DON (Gallo et al., 2020; Catellani et al., 2023), ZEN (Seeling et al., 2006; Jovaisiene et al., 2016; Catellani et al., 2025), and FUM (Gallo et al., 2020; Hartinger et al., 2022) recently reported a negative impact on cows' performances at the threshold of 500 to 1000 ppb, 300 to 350 ppb and 1000 to 2000 ppb, respectively. Based on the field aforementioned contamination levels and diet inclusions reported for specific forage in Gallo et al. (2022), non-corn forages could contribute to $5.95 \pm 6.5\%$, $15.34 \pm 8.76\%$ and $10.52 \pm 6.34\%$ of the

proposed risky threshold for ZEN, DON and FUM, respectively. These values could be increased up to $52.34 \pm 5.72\%$ for DON on 2018-2019, to $29.64 \pm 3.42\%$ for ZEN on 2024-2025 or to $26.26 \pm 7.89\%$ for FB on 2021-2022 harvest seasons, based on mean concentrations reported in Figure 1. Therefore, the *Fusarium*-produced mycotoxin contaminations should contribute to final diet contamination, when they are mixed with other contaminated corn-forages (i.e., corn silage, high moisture ear corn or high moisture corn) and concentrates. Our results also support the integration of forage-specific data into predictive models and early-warning systems by capturing occurrence patterns, environmental drivers, and the frequent co-occurrence of multiple toxins (Gallo et al., 2022). Data derived from large-scale screening further provides the basis for developing predictive equations that improve risk assessment analysis in dairy farm system and ultimately complements chemical characterization during the screening phase, increasing both efficiency and decision-making accuracy (Battilani, 2016). Integrating these aspects into recently proposed animal nutrition models (e.g., NASEM 2021, INRA 2018, NorFor 2011, and CNCPS v.6.5, as described in Van Amburgh et al., 2015) is crucial, as it allows the representation of both nutritional quality and contamination risk. Assessing mycotoxin risk shouldn't focus solely on corn silage; cereal straws and alternative silages also deserve attention, especially in years favorable to *Fusarium*. Broadening surveillance to the full forage spectrum enables a more accurate exposure assessment and better protects both animal health and milk quality.

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Table 1. Occurrence (% of positive samples) and contamination levels in non-corn forages (period: 2018–2025). The results are expressed as average values in positive samples $\pm$ standard deviation. The median and maximum concentration values are also reported

Mycotoxin	Forage type	N° samples	%Positive (>LOQ) ¹	Average (µg/kg DM)	Median (µg/kg DM)	Max. (µg/kg DM)
ZEN	All	540	29%	128 $\pm$ 151	68	943
	Mixed cereal silages ³	270	21%	83 $\pm$ 83	45	380
	Sorghum silage	76	66%	181 $\pm$ 177	124	840
	Legume silage	18	56%	61 $\pm$ 28	60	122
	Grass hay	72	24%	135 $\pm$ 169	64	598
	Straw	55	27%	180 $\pm$ 235	107	943
	Legume hay	49	20%	99 $\pm$ 107	44	335
	All	903	48%	929 $\pm$ 3037	281	46000
DON	Mixed cereal silages	452	39%	1358 $\pm$ 4420	292	46000
	Sorghum silage	108	44%	499 $\pm$ 526	283	2676
	Legume silage	37	43%	221 $\pm$ 113	191	529
	Grass hay	135	53%	403 $\pm$ 539	227	3147
	Straw	119	84%	950 $\pm$ 2130	319	16486
	Legume hay	52	44%	551 $\pm$ 638	456	3182
	All	491	20%	1005 $\pm$ 697	765	4501
	Mixed cereal silages	253	17%	1092 $\pm$ 867	824	4501
Total FUM	Sorghum silage	70	33%	1024 $\pm$ 619	790	3182
	Legume silage	26	23%	1115 $\pm$ 792	783	2673
	Grass hay	47	13%	796 $\pm$ 314	655	1395
	Straw	73	25%	801 $\pm$ 302	687	1727
	Legume hay	22	5%	1098	NA ²	1098

¹LOQ values are the following: ZEN >25 µg/kg; DON >100 µg/kg; Total FUM >500 µg/kg.

²NA = not available.

³Silages produced from autumn-winter forage mixtures, mainly composed of small-grain cereals (wheat, triticale, barley, and ryegrass).

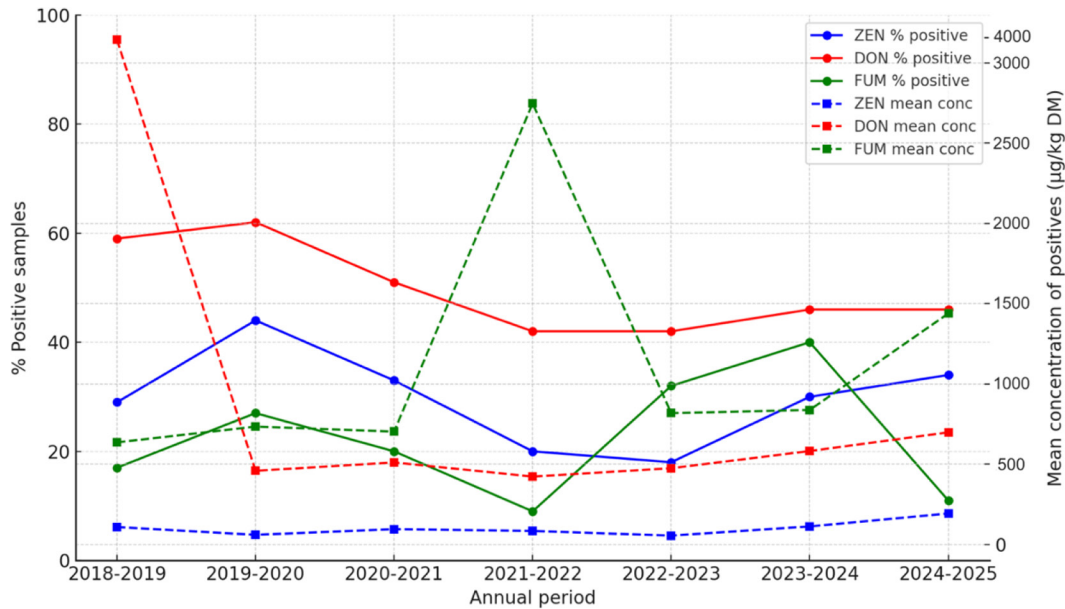


Figure 1. Annual trends in the prevalence (% positive) and mean concentration ($\mu\text{g/kg DM}$) of zearalenone (ZEN), deoxynivalenol (DON), and total fumonisins (FUM) in non-corn forages analyzed from 2018 to 2025. Solid lines indicate % of positive samples above the LOQ, and dashed lines indicate mean concentration of positive samples.

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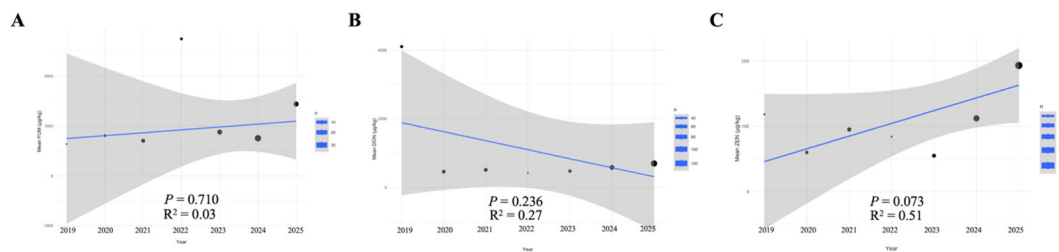


Figure 2. Weighted regression curves for each mycotoxin group (A = total FUM; B = DON; C = ZEN) and resulting from WLR statistical approach.

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Notes

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The graphical abstract has been generated using the design program Mind the Graph (accessed October 8, 2025).

Abbreviations: DON = deoxynivalenol; LC-MS/MS = liquid chromatography-tandem mass spectrometry; FUM = fumonisins; ZEN = zearalenone.