Journal of Equine Veterinary Science 151 (2025) 105632

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.elsevier.com/locate/jevs

Exposure of horses to biotoxins, phytoestrogens, and pesticides from different feed materials and supplementary feeds

L.M. Kwaß [a,†], R. Khiaosa-ard [a,†], Q. Zebeli [a,*], M. Sulyok [b], V. Milojevic [c], B.U. Metzler-Zebeli [d]

[a] Centre for Animal Nutrition and Welfare, Clinical Department for Farm Animals and Food System Science, University of Veterinary Medicine Vienna, 1210 Vienna, Austria
[b] BOKU University, Department of Agricultural Sciences, Institute of Bioanalytics and Agro-Metabolomics, Konrad-Lorenz-Strasse 20, 3430 Tulln an der Donau, Austria
[c] Sandgrueb-Stiftung, 8132 Egg bei Zürich, Switzerland
[d] Centre for Veterinary Systems Transformation and Sustainability, Clinical Department for Farm Animals and Food System Science, University of Veterinary Medicine Vienna, 1210 Vienna, Austria

ARTICLE INFO

Keywords:
Ergot alkaloids
Horse feeding
Horse health
Mycotoxins
Veterinary drug residues

ABSTRACT

Background: The occurrence of biotoxins and chemical residues in marketed horse feeds has direct influences on horse health but has not been studied yet.
Aims/objectives: The study investigated the exposure and health implications of contaminants in various horse feedstuffs available on the European market.
Methods: A total of 108 feed samples representing diverse product categories such as hay, processed roughage products, grains, and various supplementary feeds were collected from different European countries and analyzed for contaminants, including mycotoxins, phytoestrogens, pesticides, and veterinary drug residues using liquid chromatography-tandem mass spectrometry (LC-MS/MS).
Results: Findings revealed that nearly all samples contained multiple fungal metabolites, with *Fusarium* toxins being the most prevalent. Processed roughage products (e.g., cobs, cubes, flakes and pellets) containing lucerne exhibited high concentrations of phytoestrogens and plant toxins compared to hay. The data also showed that supplementary feeds, particularly grain-based mueslis and mashes, were more prevalent sources of pesticide and veterinary drug residues than feed materials. Unusual substances in horse feed like colchicine and monensin, both highly toxic to horses, were also detected in roughly 10 to 20 % of the samples. However, our risk assessement suggests that the contamination of both compounds would not pose an acute health risk to horses.
Conclusions: The study reveals the complexity of biotoxins and chemical residues and their potential risks in marketed equine feeds and underscores the critical need for targeted regulations, routine testing to ensure equine health and welfare.

1. Introduction

The equine feed market offers large variety of products and specialized feeds, tailored to the nutritional needs of various horse categories. Besides grains and roughage products, there is a large variety of supplementary feeds in the equine market including diverse grain-free products, with or without feed additives. Whilst feedstuffs provide essential nutrients to the animal, they can also act as carriers of various contaminants, originating either from raw materials or coming during feed production, processing or storage [1,2].

Feed contaminants can be of biotic origin with fungal and bacterial toxins as the most common [3–5]. Feeds can also be prone to abiotic contaminants including pesticide and veterinary drug residues [6,7]. The exposure of horses to such contaminants may threaten their performance, reproduction and health. For example, several *Fusarium*-derived mycotoxins such as deoxynivalenol (DON), zearelanone (ZEN) and fumonisin (FUM) are reported for their negative health impacts in horses [8–10]. Ergot alkaloids, a group of naturally occurring compounds produced by certain fungi, particularly the *Claviceps* and *Neotyphodium* species, pose another risk to horse health [11,12].

Besides mycotoxins, horses are exposed to several other biotoxins in their feedstuffs. Bacterial toxins like cereulide, monactin, and nonactin

* Corresponding author.
E-mail address: qendrim.zebeli@vetmeduni.ac.at (Q. Zebeli).
† Contributed equally to this work.

https://doi.org/10.1016/j.jevs.2025.105632
Received 26 February 2025; Received in revised form 14 May 2025; Accepted 15 June 2025
Available online 16 June 2025

have been detected in feeds [13], including horse pastures [6] and silage [14], though their effects, particularly on horses, remain poorly understood. Plant toxins, such as colchicine produced by meadow saffron (*Colchicum autumnale*), are known to be highly toxic to horses [15]. Studies indicate that *C. autumnale* is relatively frequent in grasslands [16], which may contaminate both hay and products containing roughages. White clover can also contain high concentrations of cyanogenic glycosides such as linamarin and lotaustralin, posing a significant concern for grazing horses, too [17,18].

Studies on pesticide and veterinary drug residues are rare in horse feeds. Son et al. [6] reported the presence of a "cocktail" of five different pesticide residues (mainly fungicides) in horse pastures, likely due to airborne contamination on pasture fields. Research in cattle feeds has also shown that over 60 % of samples contained mixtures of two to six residues/sample of pesticides and veterinary drugs including monensin [7], which is highly toxic to horses [19], and may derive from carry-over between different product lines in feed factories. This underscores the need for comprehensive research to explore potential contaminants and evaluate exposure of horses to wide range of feeds including feed materials and industrially produced supplementary feeds, as well as their implications for horse health.

To address this gap of knowledge, the current study elucidated the occurrence of various groups of contaminants in a broad range of equine feeds, consisting of both a feed materials and supplementary feeds available on the market in different European countries. We focused on the association between the presence and concentration of contaminants and feed types, specific ingredients. Our hypotheses were i) cereal grain-based feeds contain higher loads of mycotoxins and pesticides than roughage products due to their different cultivation conditions and crop characteristics, and ii) supplementary feeds (such as muesli and mash) have higher contaminant loads than single feed materials (such as cereal grains and byproducts). In addition, based on the concentration and presumed amounts the feedstuffs are consumed, the present study estimated the exposure risks of horses of important contaminants and toxins in horse nutrition.

2. Materials and methods

2.1. Feed sampling

For this study, a total of 108 single and compound horse feeds available on the European equine market were randomly collected from ready-to-feed products of various local commercial providers in Germany, Austria, France, Switzerland, and Hungary. Table 1 summarizes information on the number of samples by product category and by country of origin. The most tested product categories were processed roughage products and grain containing or grain-based muesli due to their broad use in horse feeding as roughage replacement and supplementary feed, respectively. Sampling was carried out during the period March-December of 2023. Representative samples of the feeds were collected in plastic zipped bags, stored at room temperature in a dark place, and transferred to the laboratory within one month after sampling. For the sample preparation, the feed samples were air-dried at 65°C for 48 h. Then, the dried samples were milled to a final particle size of $\leq$ 0.5 mm, using the cutting mill (SM 300; Retsch GmbH, Haan, Germany) at 1500 rpm for approximately 1 min, and the remnants ($>$ 0.5 mm) were processed using an ultra-centrifugal mill (ZM 200; Retsch GmbH, Haan, Germany) at 10,000 rpm for approximately 30 s. Finally, 5 g ($\pm$ 0.01 g) of each homogenized feed sample was weighed into 50 mL polypropylene conical tubes (Sarstedt, Nümbrecht, Germany) and stored at - 20°C until posterior analysis targeting multiple contaminants. Official ingredient information according to the product label of each product was recorded.

Table 1
Number of tested feeds by country of origin (n = 108).

Type of feedstuff	Austria	Switzerland	Germany	France	Hungary
Hay	0	0	5	0	0
Processed roughage products[1]	3	7	10	1	3
Grains[2]	2	1	4	0	0
Herbs	0	0	6	0	0
Grain-based mash	1	2	1	0	1
Grain-free mash	1	2	4	0	1
Grain-based muesli	5	7	9	0	3
Grain-free muesli	2	3	6	0	1
Byproducts & oilseeds[3]	3	4	4	0	0
Others[4]	0	0	6	0	0

[1] Include different processed products in form of cobs, cubes, pellets and flakes, of either grasses (n = 11), lucerne (n = 8) or mixed (n = 5).
[2] Include oat (n = 4), barley (n = 2), and corn flakes (n = 1) samples.
[3] Include sugar beet pulp (n = 7), wheat bran (n = 2), soybean meal (n = 1) and linseed (n = 1).
[4] Include mineral feeds (n = 4), methylsulfonylmethane (n = 1) and treats (n = 1).

2.2. Feed analyses

The horse feed samples underwent testing to screen over 1200 different contaminants, including biotoxins, pesticides, and veterinary drugs. The full list of these compounds is presented in the previous study [13]. From the total 108 samples, 83 were screened for pesticide and veterinary drug residues. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique was employed for this purpose, utilizing 5 g of finely milled and thoroughly mixed samples. The analysis took place at the University of Natural Resources and Life Sciences Vienna (BOKU) in Tulln, Austria, using a multi-analyte based method validated for fungal metabolites, selected bacterial toxins and plant toxins, pesticides and veterinary drugs in feedstuff [13]. Briefly, 5 grams of homogenized material were extracted using 20 mL of acetonitrile/water/acetic acid 79:20:1, v/v/v and shaken for 90 min on a rotary shaker (GFL 3017, GFL; Burgwedel, Germany). Next, 500 µl of the supernatants were transferred into HPLC vials and diluted with 500 µl of acetonitrile/water/acetic acid 20:79:1, v/v/v. After appropriate mixing, 5 µL of the diluted extracts were injected into the LC–MS/MS system without further pre-treatment. Mycotoxin analysis was carried out using a 1290 Series HPLC System (Agilent, Waldbronn, Germany) coupled to a QTrap 5500 LC-MS/MS System (Applied Biosystems SCIEX, Framingham, MA) equipped with Turbo Ion Spray electrospray ionization source. Chromatographic separation was performed at 25 °C running an acidified methanol/water gradient on a Gemini® C18-column, 150 × 4.6 mm i.d., 5 µm particle size, equipped with a C18 4 × 3 mm i.d. security guard cartridge (Phenomenex, Torrance, CA, US). ESI-MS/MS data was acquired in the scheduled multiple reaction monitoring mode both in positive and negative polarity in two separate chromatographic runs. The detection window width was 40 and 46 sec in the positive and negative ionization mode, respectively. The target cycle time was 1400 msec and the MS pause time was 3 msec. Compound dependent MS/MS parameters and source of reference standards are listed in in Steiner et al. [13]. Confirmation of positive metabolite identification was carried out by the acquisition of two MS/MS signals per analyte in the time scheduled multiple reaction monitoring mode which yielded 4.0 identification points according to the European Commission decision 2002/657. In addition, retention time and ion ratio had to agree to the related values of authentic standards within 0.03 min and 30 % rel., respectively. Quantitation was based on external calibration using serial dilutions of a multi-analyte stock solution. Results were corrected for apparent recoveries determined in feed [13]. The performance of the method is verified on a continuous basis by participation in a proficiency testing scheme (BIPEA, Gennevilliers, France) with $>$ 96 % of the 2400 results submitted so far (including complex animal feed) exhibiting a z-score of $-2 < z < 2$. Limits of

detection and limits of quantification were determined according to the EURACHEM guide and are listed in Steiner et al. [13].

2.3. Data management and statistical analysis

Descriptive statistics (occurrences and concentration values of feed contaminants: average, median, minimum, and maximum) were calculated using only the positive values ($x \geq$ limit of detection (LOD)). Data below LOD were considered not detectable and we considered values above LOQ for data analysis. For the statistical analysis, the horse feeding products were grouped based on the botanical composition and feeding goal as follows: meadow hay ($n = 5$), processed roughage products (e.g., cobs, cubes, pellets and flakes) ($n = 24$), grains ($n = 7$), grain-based muesli ($n = 24$), grain-free muesli ($n = 12$), grain-based mash ($n = 5$), grain-free mash ($n = 8$), herbs ($n = 6$), byproducts and oilseeds ($n = 11$), and others ($n = 6$). The latter category included mineral feeds ($n = 4$), methylsulfonylmethane (MSM; $n = 1$) and horse treat ($n = 1$). A subset of data using only the processed roughage products was tested for the effect of roughage groups including grasses ($n = 11$), lucerne ($n = 8$) and mixed ($n = 5$), and then tested against hay ($n = 5$). All statistical analyses were performed with SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). The Means procedure was used to obtain the descriptive statistics of the whole dataset. Analysis of variance for the comparison among test groups was done using the MIXED procedure of SAS. Global F-tests were conducted to identify significant effects ($P < 0.05$), and due to the unequal sample sizes among horse feed products, the Tukey-Kramer test was used to compare their significant differences from each other. The resulting data reported are the least-squares means (LS means) and standard error of the least-squares mean (SEM). The co-contamination results (Fig. 1) were illustrated using a radar chart created using Microsoft Excel®.

3. Results

3.1. Overall occurrence and concentrations of contaminants

Contaminants were classified into 15 groups assigned to different fungal metabolites including mycotoxins, plant toxins, phytoestrogens, bacterial metabolites, lichen metabolites, plant metabolites, unspecific metabolites, and pesticide and veterinary drug residues. The fungal metabolite group comprised *Alternaria* metabolites, *Aspergillus* metabolites, *Fusarium* metabolites, *Penicillium* metabolites, ergot alkaloids, and other fungal metabolites. Table 2 shows that 11 out of 15 contaminant groups were detected in over 90 % of the horse feed samples. A total of 99 % ($n = 107$) of the samples tested positive for fungal metabolites, particularly *Fusarium* metabolites, unspecific metabolites, and pesticide residues. The only sample without any contamination was the synthetic product consisting purely of MSM. About 98 % ($n = 106$) of the horse feeds contained *Alternaria* metabolites, *Penicillium* metabolites, and other fungal metabolites. Bacterial metabolites were found in only 16 % ($n = 17$) of the samples, making this the least frequently detected group of contaminants and with the lowest mean concentration at just 0.6 µg/kg. Ergot alkaloids and lichen metabolites were also found at lower rates, 62 % and 57 % out of 108 samples, respectively. In this study, 96 % of all tested samples were detected with phytoestrogens, 90 % with plant metabolites and 94 % with plant toxins. The highest mean concentrations were observed in the groups of phytoestrogens (35 mg/kg) and plant toxins (38 mg/kg). There were 26 compounds classified as plant toxins (Table S1), with cyanogen glycosides linamarin and lotaustralin being the most frequently found compounds (70 and 88 % of the 108 samples, respectively). Colchicine was also detected in 19 % of the samples. was detected for bacterial metabolites. Veterinary drug residues were present in 20.5 % of the 83 samples tested.

3.2. Number of contaminants and co-contaminations in feed samples

Each feed product group exhibited complex co-contaminations

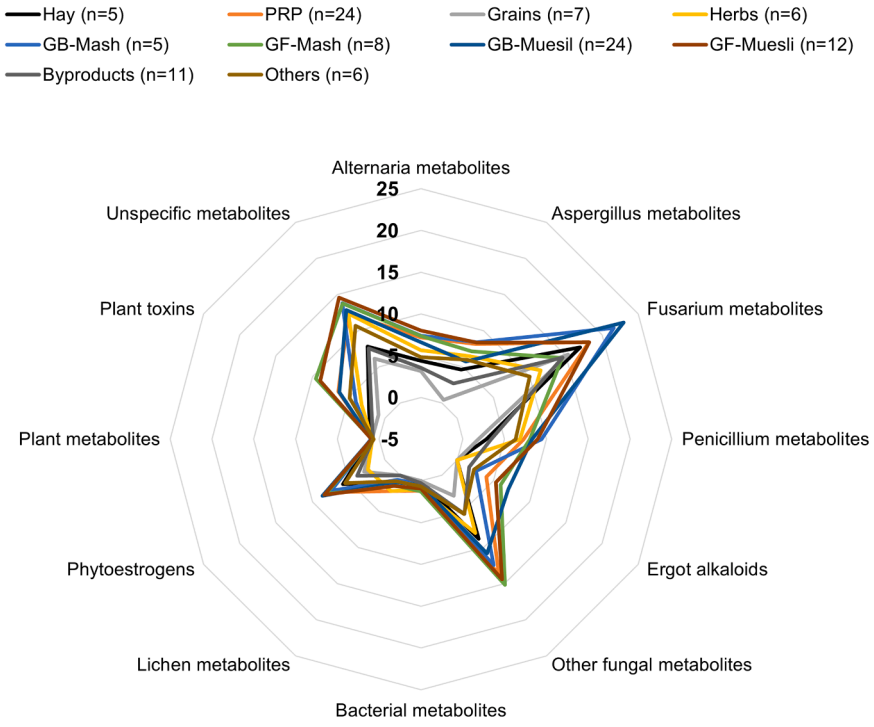

Fig. 1. Co-contamination of various product groups with mycotoxins and other biotoxins. Values presented in the plot are least-squares means and for all groups of metabolites except plant metabolites, there was a significant effect of horse feed group ($P < 0.05$). PRP = processed roughage products, GB = grain-based and GF = grain-free.

Table 2
Occurrence and concentrations of the contaminants in the analyzed equine single and supplementary feeds.

Groups	No. of samples	No. Positive	% positive	Concentration (µg/kg)			
				Mean	SD	Maximum	Median
Total fungal metabolites	108	107	99	3,030	3,295	19,224	2,095
Alternaria metabolites	108	106	98	1,056	1,745	13,574	610.0
Aspergillus metabolites	108	100	93	290	917	8,758	56.4
Fusarium metabolites	108	107	99	1,003	1,020	6,174	821
Penicillium metabolites	108	106	98	421	1,120	8,221	143
Ergot alkaloids	108	67	62	63.0	122	898.5	10.4
Other fungal metabolites	108	106	98	196	349	2,826	75.9
Bacterial metabolites	108	17	16	0.6	1.9	12.7	0.0
Lichen metabolites	108	62	57	37.6	149	1288	1.6
Phytoestrogens	108	104	96	35,055	60,754	547,595	23,337
Plant metabolites	108	97	90	1,021	1,202	5,738	683
Plant toxins	108	101	94	38,268	51,983	20,5624	10,034
Unspecific metabolites	108	107	99	1,762	2,222	16,262	1,193
Total pesticide residues	83	82	99	157	191	1,059	100
Total veterinary drug residues	83	17	20.5	122	558	4,223	0.0

(Fig. 1). Across all fungal metabolites, *Fusarium* metabolites were the most prevalent group of metabolites showing an average of 10 – 23 metabolites per sample. Other fungal metabolites (unclassified fungal origins) and unspecific metabolites (i.e., possibly deriving from multiple origins) were also more common than other metabolite groups. The smallest groups of metabolites were bacterial and plant metabolites. When comparing feed product groups, in general, processed products including roughage products (e.g., cobs and pellets) and cereal products (e.g., mashes and mueslis) displayed a more complex fungal metabolite profile than single feed items like hay and grains.

3.3. Differences of contamination concentrations between the feed product groups

Table 3 shows that *Aspergillus* (1.9 mg/kg) and *Penicillium* (2.1 mg/kg) metabolites were detected at the highest concentrations in grain-based mash samples. Plant toxins were found at the highest concentration of 96.4 mg/kg in grain-free mash samples. Other fungal metabolites had the highest concentration of 0.9 mg/kg in hay. The concentrations of mycotoxins produced by *Fusarium* fungi, specifically those mycotoxins regulated by guidance values, were significantly higher in herb products. For example, the concentration of DON was significantly higher in herb products, at 0.99 mg/kg, compared to other horse feed

Table 3
Concentration of fungal metabolites including relevant mycotoxins, bacterial metabolites, lichen metabolites, phytoestrogens, plant metabolites, plant toxins and unspecific metabolites per product group.

Groups	Concentration (µg/kg)										*P* value
	Hay (n = 5)	PRP[1] (n = 24)	Grains (n = 7)	Herbs (n = 6)	GB-Mash[2] (n = 5)	GF-Mash[2] (n = 8)	GB-Muesli[3] (n = 24)	GF-Muesli[3] (n = 12)	Byproducts (n = 11)	Others[4] (n = 6)	
Alternaria metabolites	1281	1400	502	1261	1325	589	1331	813	577	588	0.880
Aspergillus metabolites	735^{ab}	187^{b}	19.3^{b}	167^{b}	1889^{a}	411^{ab}	62.6^{b}	289^{b}	129^{b}	488^{ab}	0.015
Fusarium metabolites	1301	1154	568	1406	1803	823	949	962	575	908	0.489
Penicillium metabolites	64.1$^{(b)}$	716^{ab}	20.8^{b}	627^{ab}	2123^{a}	256^{ab}	135^{b}	243^{b}	162^{b}	578^{ab}	0.033
Ergot alkaloids	0	62.6	0	0	4.1	115	76.7	170	21.4	40.4	0.024
Other fungal metabolites	899^{a}	249^{b}	9.4^{b}	324^{ab}	236^{b}	234^{b}	102^{b}	215^{b}	25.6^{b}	47.5^{b}	<0.001
Bacterial metabolites	0	1.3	0	0	2.5	0	0.06	0.6	0.5	0.3	0.169
Lichen metabolites	22.8^{b}	45.2^{b}	0^{b}	272^{a}	22.1^{ab}	9^{b}	35.6^{b}	14.4^{b}	0^{b}	3.4^{b}	0.040
Phytoestrogens	5347	44,882	86	21,645	2371	27,412	37,420	40,796	53,472	57,449	0.548
Plant metabolites	462^{b}	2196^{a}	74.5^{b}	1977^{ab}	312^{b}	543^{b}	752^{b}	877^{b}	279^{b}	888^{ab}	<0.001
Plant toxins	1994^{b}	61,484^{ab}	12.2^{b}	1939^{b}	31,074^{ab}	96,428^{a}	27,781^{b}	67,092^{ab}	18,225^{b}	6097^{b}	<0.001
Unspecific metabolites	799	1998	129	1641	4404	1648	1386	2261	2095	1498	0.125
Specific group or individual mycotoxin											
Guidance value cate. mycotoxins	292^{b}	177^{b}	139^{b}	1003^{a}	179^{b}	230^{b}	143^{b}	140^{b}	82.3^{b}	495^{b}	<0.001
Deoxynivalenol	223^{b}	143^{b}	52.8^{b}	993^{a}	87.2^{b}	224^{b}	73.1^{b}	120^{b}	47.3^{b}	487	<0.001
Fumonisin B1+B2	5.1	6.1	5.3	0	1.7	0	12.5	7.1	0	5.5	0.430
T2+HT2 toxins	0	12.4	79.9	9.3	83.2	0	32.6	5.8	1.5	0	0.023
Zearalenone	19.8	15.8	1.8	0.7	7.5	6.1	25.6	7	33.6	2.5	0.652
Emerging mycotoxins[5]	656	618	234	340	937	365	514	492	365	345	0.350
Culmorins	31.4	96.2	70.4	0	187	52.2	176	121	137	17.3	0.253
Enniatins	60	101	33.2	14.5	56.7	58.1	66.9	40.8	54.2	43.5	0.081
Siccanol	349	217	58.7	310	473	172	82.3	209	11.3	272	0.029

The superscripts ^{ab} indicates groups that differ ($P < 0.05$) from the highest value. Pairwise comparisons were compared according to the Tukey-Kramer method.
[1] Processed roughage products, including products of hay, lucerne cubes and hay flakes.
[2] Includes products of grain-based mash or muesli.
[3] Includes products of grain-free mash or muesli.
[4] Other products include mineral feeds (n = 4), methylsulfonylmethane (n = 1) and treat (n = 1) samples.
[5] The sum of fungal metabolites categorized as emerging mycotoxins according to Penagos-Tabares et al. [3].

groups. Other *Fusarium*-produced mycotoxins showed no significant differences among different types of horse feeds. Plant metabolites were found in the highest concentration in processed roughage products and significantly lower in grains and byproducts. The highest concentration of plant toxins was detected in grain-free mash group. On average, phytoestrogens showed the lowest concentration in grains, although there was no significant difference among the feed product groups (Table 3).

The comparisons among the roughage groups, based on their botanical composition (hay or processed roughage products based either on grass, lucerne or mixed) are shown in Table 4. While ergot alkaloids were not found in hay samples, they were detected at the highest concentration of 0.1 mg/kg in grass-based processed roughage products. In lucerne-based and mixed roughage products, the concentration of ergot alkaloids was not significantly different from other groups. Other fungal metabolites were the only contaminants detected at the highest concentration of 0.9 mg/kg in hay, with significantly lower concentrations in mixed-based plant products. Phytoestrogens were found at significantly higher concentrations in lucerne and mixed-based roughage products (60.9 mg/kg) than in hay samples. Plant toxins were also observed at the highest concentration of 113.6 mg/kg in mixed products and differed significantly to both lucerne and hay-based samples.

3.4. Occurrences and concentrations of abiotic contaminants

Table 5 shows the data of the concentration and occurrence of pesticides and veterinary drug residues in the analyzed 83 samples. The concentrations of pesticides and veterinary drug residues were not different among groups. Fungicide residues were the most frequent type of pesticide residues detected in the feed samples. Products like mashes and muesli, regardless of whether grain-free or not, contained significantly higher number of pesticides per sample compared to the analyzed processed roughage products, grains, and byproducts (Table 5). The number of insecticide residues (3.4 residues/sample) and fungicide residues (7.4 residue/sample) was most frequent in grain-based mash samples, leading to the highest number of total pesticides (10.8 residue/sample). Considering individual pesticide and veterinary drug residues, piperonyl butoxide, tebuconazole, and didecyldimethylammonium chloride were the most frequently found pesticides. The concentration of piperonyl butoxide – a pesticide potentiator – was significantly higher in muesli products containing grains compared to processed roughage products, but did not differ with other groups. The average concentration of piperonyl butoxide was 0.1 mg/kg in grain-based muesli and 0.08 mg/kg in grain-based mash products. Monensin and dinitrocarbanilide were the most detected veterinary drug residues in the samples. Monensin was detected in 10.8 % (9 out of 83) of all analyzed samples, with concentrations varying from 9 to 1897 mg/kg.

4. Discussion

In this study, we screened for over 1200 compounds. We detected a broad spectrum (>250 compounds) of biotoxins and chemical contaminants in a broad range of commercial feedstuffs for horses available on the market in five different European countries.

4.1. Exposure risks of horses towards biotoxins in the feed

Our study showed that nearly all tested feed samples were contaminated with numerous fungal metabolites, including multiple mycotoxins, particularly from *Fusarium, Alternaria*, and *Penicillium* fungi, suggesting a consistent susceptibility of horse feeds to fungal contamination, and respectively a probable exposure of animals to these biotoxins. Of all metabolite groups, *Fusarium* metabolites were the most frequent contaminants across all types of feedstuffs analyzed. These findings echo previous observations reporting FUM and DON as the most prevalent in horse feeds, particularly in grains such as corn and oats [20]. Our findings also align with reports indicating occurrence of mycotoxins in over 80 % of hay samples in the UK, in which Durham [8] detected FUM B1, DON, and ZEN in hay samples exclusively with cases of liver disease outbreaks.

The EU legislation (2006/576/EC and Commission Recommendation 2016/1319) provides the guidance value for DON, ZEN, ochratoxin A, T-2- and HT-2 toxin, and FUM in feed and pet food. Of these, FUM B1+B2 are the only mycotoxins with a guidance level set for horses (i.e., 5 mg/kg of FUM B1+B2 in feed at a 12 % moisture content). This is because of documented links between FUM B1 and equine leukoencephalomalacia [20,21]. To assess the exposure risk of feed contaminants, we focused on the maximum concentration of a contaminants detected in our surveyed samples. Accordingly, the highest FUM concentration found was 0.052 mg/kg in a grain product, which is well below the set guidance value, suggesting sufficient safety of horse feeds analyzed in this study in relation to FUM toxicity.

The DON and ZEN, on the other hand, do not have a maximum allowable concentration for horses. The highest DON concentration permitted in swine feed is 0.9 mg/kg, and for young ruminants, it is 2 mg/kg. In our study, we found one processed roughage sample and one herb sample having DON concentrations exceeding the guidance level set for young ruminants, signaling the possibility for exposure to DON in horses. However, horses are reported to have a generally high tolerance to DON [22]. The latter authors showed that the daily intake up to 9.5

Table 4
Concentration of grouped fungal metabolites, bacterial metabolites, lichen metabolites, phytoestrogens, plant metabolites, plant toxins and unspecific metabolites in hay and processed roughage products.

		Processed roughage products				
Groups (µg/kg)	Hay ($n = 5$)	Grass[1] ($n = 11$)	Lucerne[2] ($n = 8$)	Mix[3] ($n = 5$)	SE	P value
Alternaria metabolites	1281	2443	383	733	390.3	0.311
Aspergillus metabolites	736	147	194	264	117.5	0.412
Fusarium metabolites	1301	1452	744	1153	131.9	0.706
Penicillium metabolites	64.1	1194	304	321	215.0	0.439
Ergot alkaloids	0[b]	102.7[a]	28.1[ab]	29.5[ab]	19.0	0.020
Other fungal metabolites	899[a]	299[ab]	162[b]	280[ab]	143.8	0.054
Bacterial metabolites	0	2.1	0.9	0.2	0.4	0.346
Lichen metabolites	22.8	18.4	25.4	135.5	24.5	0.271
Phytoestrogens	5347[b]	34,522[ab]	49,091[ab]	60,939[a]	10,388	0.045
Plant metabolites	462[b]	1184[b]	3884[a]	1725[b]	638.3	<0.001
Plant toxins	1994[b]	54,477[ab]	38,545[ab]	113,599[a]	20,127	0.017
Unspecific metabolites	800	2440	1286	2165	330.1	0.143

[ab] Least-squares means within the same row sharing no common superscripts differ at $P < 0.05$ according to Tukey Kramer test.
[1] Includes hay cobs, cubes, and flakes.
[2] Includes lucerne cobs and pellets.
[3] Includes products based on a mix of grasses and lucerne.

Table 5
Concentration and occurrence of pesticide, pesticide potentiator, and veterinary drug residues per product group.

Groups	Feed products tested for pesticide and veterinary drug residues							P value
	PRP[1] (*n* = 22)	Grains (*n* = 6)	GB-Mash[2] (*n* = 5)	GF-Mash[3] (*n* = 6)	GB-Muesli[2] (*n* = 24)	GF-Muesli[3] (*n* = 11)	Byproducts[4] (*n* = 9)	
Concentration (µg/kg)								
Insecticide residues	34.3	40.9	97.4	19.1	121.4	38.2	29.3	0.318
Fungicide residues	44.8	35.9	193	152	116.7	71.3	82.4	0.113
Herbicide residues	9.1	0	0	11.6	8.3	0	0	0.828
Total pesticide residues	88.2	76.8	291	182	246	109	111	0.039
Vet drug residues	111.6	0	303	0	81.2	384	0	0.678
Most frequently detected residues (>60 % occurrence of all samples)								
Piperonylbutoxide	5.4[b]	38.1[ab]	80.9[ab]	6.7[ab]	102.3[a]	13.1[ab]	17.6[ab]	0.047
Didecyldimethylammoniumchloride	14	3.3	8.8	14.7	15.9	10.2	8	0.732
Tebuconazol	4.6	6.9	21	35.7	34.3	11.5	3	0.400
Detected number per sample of:								
Insecticide residues	0.6[c]	1.2[b]	3.4[a]	1.3[b]	2.2[ab]	2.5[ab]	1.3[b]	<0.001
Fungicide residues	1.9[b]	0.8[b]	7.4[a]	6.7[a]	4.4[a]	4.4[ab]	2.9[ab]	<0.001
Herbicide residues	0.4	0	0	0.2	0.1	0	0	0.056
All pesticides	2.9[b]	2.0[b]	10.8[a]	8.2[a]	6.7[a]	6.8[a]	4.2[b]	<0.001
Veterinary drugs	0.3	0	1	0	0.6	0.6	0	0.392

[ab] Least-squares means within the same row sharing no common superscripts differ at $P < 0.05$ according to Tukey Kramer test.
[1] Processed roughage products, including products of grass and lucerne cobs, cubes, flakes and pellets.
[2] Includes products containing grains.
[3] Includes products of grain-free mash or muesli.
[4] Includes sugar beet pulp ($n = 6$), wheat bran ($n = 2$) and soybean meal ($n = 1$).

mg/100 kg body weight (BW) did not lead to adverse clinical signs or major changes in immune response in mares.

The highest ZEN concentration we found was about 0.252 mg/kg in a grain product and sugar beet pulp product, which was just above the permitted limit for sows (0.25 mg/kg), and half of that permitted for dairy cows. Horses exhibit a lower sensitivity to ZEN compared to pigs, because they metabolize ZEN primarily into β-zearalenol [10], in contrast to swine [23] or ruminants [24], where the highly estrogenic α-zearalenol is the dominant metabolite. An acute toxicity from consuming such commercial products would be unlikely in horses, although the chronic exposure to ZEN, even at low doses, can influence reproductive parameters in mares, such as progesterone levels and pregnancy rates [25].

It has to be highlighted that about half of the samples in our study were co-contaminated with DON and ZEN. Schumann et al. [9] demonstrated that DON and ZEN, along with their metabolites, could significantly reduce the viability and proliferation of equine immune cells *in vitro*. Given the complexity of *Fusarium* metabolite profiles in horse feed products shown in the present study, it is imperative to raise concerns about potential synergistic effects among contaminants due to the complex interactions between fusariotoxins and other compounds, which may exacerbate their toxic effects [26]. To the best of our knowledge, no study has yet investigated the impact of co-contamination with multiple toxins on horses. Ensley and Mostrom [20] emphasized the lack of dose-response data for many mycotoxins in equine diets, suggesting a need for further studies on the synergistic effects of these contaminants.

Another highlight of the present work was evidence of plant toxins, especially colchicine, in a significantly higher concentration in grain-free mashes than in other horse feeds. Colchicine is a toxin found in the well-known toxic plant – meadow saffron (*Colchicum autumnale*). A dose of 1 mg of colchicine per kg of BW can be lethal for a horse [15,27]. In this study, none of the five hay samples contained colchicine, but 20.4 % of the other 103 horse feed products did. The highest concentration found was 29.6 mg/kg in a mash containing among others grass meal from a Hungarian supplier (Table S2), which has a feeding recommendation of 50–200 g per 100 kg of BW. The second-highest concentration detected was 8.4 mg/kg in a processed roughage product (hay cobs) from the same Hungarian supplier, intended to be fed at a rate of 1000–1500 g per 100 kg of BW. Another notably high concentration was 4.7 mg/kg was observed in a grain-free muesli product, again from Hungary, which is recommended to be fed at 200–400 g per 100 kg of BW. All other samples contained colchicine at concentrations below 0.05 mg/kg of feed. As assesed in Table S2, these concentrations should not pose a direct lethal risk to a horse's health, as even the two products with the highest concentrations are significantly below the lethal dose and below one-quarter of the dose known to cause diarrhea [15]. However, in regions where meadow saffron is widespread, it may be difficult to avoid feeding hay contaminated with this toxic plant, but in addition, as shown in our study, other feed sources can as well contain colchicine. Altogether, this raises concerns about its accumulation in feed rations and the possibility for acute effects on horse health.

4.2. Exposure and risks of horses towards pesticides and drugs in feeds

The most frequently detected pesticide residues were piperonyl butoxide, didecyldimethylammonium chloride and tebuconazole, which were present in 65 % of the 83 samples analyzed. The pesticide residues were more prevalent in processed products, especially grain-based mash and muesli products. Based on these findings, pesticides are considered as common in marketed feeds, which suggests that horses chronically consume these pesticide residues. This hints the need for toxicity research and especially on these three residues. The specific effects of these pesticides on horses are yet to be investigated. Plumlee [28] noted that while many herbicides have low direct toxicity effect in mammals, they can indirectly cause toxicosis by altering the toxicity of poisonous plants. Also, there are indications that applying herbicides on pastures can increase palatability of certain poisonous plants, especially during droughts, making them more palatable to animals [29]. This insight is particularly relevant given the presence plant-based contaminants seen in our study.

Overall, veterinary drug residues were rarely detected, with monensin and dinitrocarbanilide being the most common, found in 10.8 % of all samples (5 samples from Hungary, 3 from Austria and 1 from Switzerland) from different suppliers. Interestingly, both residues were also found in cattle feed samples earlier [6]. Monensin is an anticoccidial drug authorized as a feed additive for poultry but not for cattle in the EU. Horses are much more sensitive to it, with symptoms like anorexia, ataxia and sweating, with a median lethal dose of 2–3 mg/kg of BW [19]. The most likely explanation for horse feed contamination in the current study is that during the manufacturing process residues of monensin were transferred from poultry feed to horse feed that was subsequently

produced using same equipment. This likely cross-contamination of monensin from poultry feeds during production suggests a need for more rigorous segregation practices in feed manufacturing. Legal regulations (EC/2009/8) permit up to 1.25 mg/kg of monensin in horse feed with a moisture content of 12 %. The highest concentration detected in this study was 1.9 mg/kg in a grain-free muesli product from Hungary (Table S3), suggesting that marketed products can have the contamination exceeding the threshold. Respectively, we could also assesed the potential exposure risk to horses, which can be fed, as per manufacturers' recommendation, 200–400 g of this product per 100 kg of BW. Two other products from the same supplier from Hungary contained 0.2 mg/kg of monensin: one was a muesli, recommended to be fed at 400–1200 g per 100 kg of body weight, and the other was a grain-containing mash, with feeding instructions of 50–200 g per 100 kg of BW. As shown in Table S3, none of these levels of intake would be lethal for horses.

4.3. Comparison among feed products revealing risky feed sources in the horse diets

We found that single-ingredient feeds, like hay, grains, and byproducts, generally have a lower concentration and number of mycotoxins and pesticide residues than supplementary feeds. The later blend diverse ingredients and undergo more processing steps than single feeds which appears to increase the contamination risk. Still, without the detailed ingredient composition, it was not possible to identify specific ingredients of supplementary feeds that trigger the occurrence of specific contaminants. Respectively, supplementary feeds tend to have more complex profiles of fungal contaminants compared to single feedstuffs. Also, the moisture content of the different ingredients of compound feed might differ and therefore positively enhance fungal growth. This emphasizes the need for further consideration regarding horse feed composition and production processes. Further, the complexity of supplementary feeds may increase the risk of mycotoxin contamination. This might be the case because each ingredient can introduce its own set of contaminants. Additionally, the blending process can inadvertently spread contaminants from one ingredient to others. On the other hand, single ingredient products often originate from one source making quality controls easier and the process of production less complicated. This considerably reduces the risk of contamination. Still, if single products are contaminated but not controlled for contamination, it may result in ingestion of high or even lethal doses of certain toxicants.

In this study, we found significantly higher concentrations of ergot alkaloids in grain-free muesli than in the other feedstuffs. We also observed that grass-based processed roughage products exhibited a greater number of ergot alkaloids and fungal metabolites other than *Alternaria, Aspergillus, Fusarium*, and *Penicillium* species, suggesting that grain-free muesli and grass-based processed products are potential dietary risk sources of ergot alkaloid exposure in horses. Besides *Claviceps* spp., which produce ergot alkaloids and are commonly associated with grains like rye and triticale [6], other fungal endophytes, like *Epichloe* spp., which are more often found on grasses (i.e., *Fescue* spp. and *Lolium* spp.), may also have contributed to the ergot alkaloid contamination in the grain-free muesli and grass-based products in this study [29,30]. Previous studies have also reported higher concentrations of ergot alkaloids in grass-based forages compared to hay and lucerne-based products [11,12].

Our study found grain-based mash to contain a significantly higher concentration of *Aspergillus* and *Penicillium* metabolites, but not those of *Fusarium*, than other analyzed horse feeds. These fungal species proliferate during storage (i.e., storage mold), while *Fusarium* species prefer more moist conditions and so thrive in the field. It might be that some ingredients, such as molasses, added to mash can retain higher moisture and are more readily accessible to *Aspergillus and Penicillium* species during storage. In our study, all the mash products tested also contained oilseed components (especially from flax) as major ingredients. Flaxseed products that are processed (e.g. ground or cracked) in order to increase nutrient accessibility, also render them being prone to lipid oxidation and fungal growth [31].

When comparing roughage feed, we observed that processed roughage products, especially those made of lucerne, in general exhibit higher concentrations of plant metabolites, phytoestrogens, and plant toxins than hay. Wyse et al. [32] highlighted that phytoestrogens are prevalent in legume species, which are common in equine diets. but different factors in addition to plant species can lead to high concentrations of phytoestrogens in forage [5,6]. Phytoestrogens are known to lead to reproductive issues including uterine edema and anovulation in mares, depending on concentration and exposure duration [32].

Our study identified another contamination source being the herbs, which showed relatively high concentrations of all guidance value mycotoxins as compared to other feed groups. The reason for that might be that moisture as a causative factor for fungal growth can be retained if the herbs have broad leaves and dense growth patterns. Moreover, herbs are often air dried and do not undergo other processing treatment, namely any additional heat treatments.

Lastly, we observed significantly higher number of pesticides, especially insecticides and fungicides in compound feeds including all mashes and mueslis. This again might be because of the higher complexity of ingredients in supplementary feeds and multiple possible contaminations and cross-contaminations of each ingredient. The detection of pesticide residues, such as piperonyl butoxide and tebuconazole, in our study aligns with the broader concerns about pesticide toxicosis in horses [28,33].

It must be noted that despite the wide range of compounds screened in the current study, there are compounds that could be relevant to horse health like botulinus toxin, cantharidin, and slaframine but were not covered by the current method. The last compound is not compatible with the LC conditions. Therefore, the current study cannot assess the contamination and their risk of these compounds on horse health.

5. Conclusion

To sum up, over 90 % of 108 analyzed horse feed samples in this study showed various co-contaminations with single and multiple contaminants of biotic and abiotic origin. The most frequent contaminant groups overall were mycotoxins and pesticide residues. In general, single feedstuffs showed lower concentrations and number of mycotoxins and pesticide residues than compound feeds, whereas processed roughage products had higher concentrations of plant metabolites, phytoestrogens, and plant toxins than hay. More specifically, some niche products like herbs and grain-free muesli also showed elevated mycotoxin contaminations. This observation should be considered especially when given such products to health-compromised horses. Nevertheless, we were not able to determine specific ingredients being responsible for a higher contamination load of the supplementary feeds. None of the horse feeds in this study seemed to have a critical concentration of contaminants known from literature to have adverse acute impacts on horse`s health. However, every product showed a complex set of contaminants. The risk of such co-contamination is not known but must be assessed by future research. This study highlights an urgent necessity for research into the effects of co-contaminants on equine health, especially for the cumulative impacts of low-dose mycotoxins, plant toxins, and pesticides. The outcomes of such research could significantly inform safe feeding practices and regulatory standards.

Financial disclosure

The feed sampling and analyses were financially supported by Sandgrueb-Stiftung. Other than that, this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

New submission at journal of equine veterinary science: ethics in publishing

I also like to highlight that work described in this article has been carried out in accordance with code of ethics, following the ethical guidelines stated in Elsevier's Publishing Ethics Policy.

CRediT authorship contribution statement

L.M. Kwaß: Writing – review & editing, Writing – original draft, Visualization, Investigation. **R. Khiaosa-ard:** Writing – review & editing, Visualization, Validation, Formal analysis, Data curation. **Q. Zebeli:** Writing – review & editing, Supervision, Project administration, Conceptualization. **M. Sulyok:** Writing – review & editing, Software, Resources, Investigation. **V. Milojevic:** Writing – review & editing, Methodology, Investigation, Conceptualization. **B.U. Metzler-Zebeli:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

I like to highlight that none of the authors has any conflict of interest with this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jevs.2025.105632.

References

[1] Kamphues J. Feed hygiene and related disorders in horses. editors. In: Geor RJ, Harris PA, Coenen M, editors. Equine applied and clinical nutrition. Philadelphia: W.B. Saunders; 2013. p. 367–80.

[2] Bishop R. The manufacturers' role in feed quality and safety: a discussion on methods used in feed manufacturing processes to assure feed hygiene and safety. editors. In: Geor RJ, Harris PA, Coenen M, editors. Equine applied and clinical nutrition. Philadelphia: W.B. Saunders; 2013. p. 381–92.

[3] Liesener K, Curtui V, Dietrich R, Märtlbauer E, Usleber E. Mycotoxins in horse feed. Mycotoxin Res 2010;26:23–30. https://doi.org/10.1007/s12550-009-0037-8.

[4] Streit E, Schatzmayr G, Tassis P, Tzika E, Marin D, Taranu I, Tabuc C, Nicolau A, Aprodu I, Puel O, Oswald IP. Current situation of mycotoxin contamination and co-occurrence in animal feed–focus on Europe. Toxins 2012;4:788–809. https://doi.org/10.3390/toxins4100788.

[5] Penagos-Tabares F, Khiaosa-ard R, Nagl V, Faas J, Jenkins T, Sulyok M, Zebeli Q. Mycotoxins, phytoestrogens and other secondary metabolites in Austrian pastures: occurrences, contamination levels and implications of geo-climatic factors. Toxins 2021;13:460. https://doi.org/10.3390/toxins13070460.

[6] Son V, Penagos-Tabares F, Hollmann M, Khiaosa-ard R, Sulyok M, Krska R, Zebeli Q. Changes in the nutrient profile and the load of mycotoxins, phytoestrogens, and pesticides in horse pastures during spring and summer in Austria. J Equine Vet Sci 2023;131:104958. https://doi.org/10.1016/j.jevs.2023.104958.

[7] Penagos-Tabares F, Sulyok M, Faas J, Krska R, Khiaosa-ard R, Zebeli Q. Residues of pesticides and veterinary drugs in diets of dairy cattle from conventional and organic farms in Austria. Environ Pollut 2023;316:120626. https://doi.org/10.1016/j.envpol.2022.120626.

[8] Durham AE. Association between forage mycotoxins and liver disease in horses. J Vet Intern Med 2022;36:1502–7. https://doi.org/10.1111/jvim.16486.

[9] Schumann B, Winkler J, Mickenautsch N, Warnken T, Dänicke S. Effects of deoxynivalenol (DON), zearalenone (ZEN), and related metabolites on equine peripheral blood mononuclear cells (PBMC) in vitro and background occurrence of these toxins in horses. Mycotoxin Res 2016;32:153–61. https://doi.org/10.1007/s12550-016-0250-1.

[10] Songsermsakul P, Böhm J, Aurich C, Zentek J, Razzazi-Fazeli E. The levels of zearalenone and its metabolites in plasma, urine and faeces of horses fed with naturally, fusarium toxin–contaminated oats. J Anim Physiol Anim Nutr 2013;97:155–61. https://doi.org/10.1111/j.1439-0396.2011.01253.x.

[11] Putnam MR, Bransby DI, Schumacher J, Boosinger TR, Bush L, Shelby RA, Vaughan JT, Ball D, Brendemuehl JP. Effects of the fungal endophyte acremonium coenophialum in fescue on pregnant mares and foal viability. Am J Vet Res 1991;52:2071–4. https://doi.org/10.2460/ajvr.1991.52.12.2071.

[12] Coufal-Majewski S, Stanford K, McAllister T, Blakley B, McKinnon J, Chaves AV, Wang Y. Impacts of cereal ergot in food animal production. Front Vet Sci 2016;3:15. https://doi.org/10.3389/fvets.2016.00015.

[13] Steiner D, Sulyok M, Malachová A, Mueller A, Krska R. Realizing the simultaneous liquid chromatography-tandem mass spectrometry based quantification of >1200 biotoxins, pesticides and veterinary drugs in complex feed. J Chromatogr A 2020;1629:461502. https://doi.org/10.1016/j.chroma.2020.461502.

[14] Shimshoni JA, Cuneah O, Sulyok M, Krska R, Galon N, Sharir B, Shlosberg A. Mycotoxins in corn and wheat silage in Israel. Food Addit Contam Part A 2013;30:1614–25. https://doi.org/10.1080/19440049.2013.802840.

[15] Humphreys DJ. Veterinary toxicology. 3rd ed. London: Baillière Tindall, London; 1988. p. 154–78.

[16] Winter S, Penker M, Kriechbaum M. Integrating farmers' knowledge on toxic plants and grassland management: a case study on Colchicum autumnale in Austria. Biodivers Conserv 2011;20:1763–87. https://doi.org/10.1007/s10531-011-0060-x.

[17] McGorum BC, Anderson RA. Biomarkers of exposure to cyanogens in horses with grass sickness. Vet Rec 2002;151:442–5. https://doi.org/10.1136/vr.151.15.442.

[18] McGorum BC, Pirie RS, Fry SC. Quantification of cyanogenic glycosides in white clover (Trifolium repens L.) from horse pastures in relation to equine grass sickness. Grass Forage Sci 2012;67:274–9. https://doi.org/10.1111/j.1365-2494.2011.00844.x.

[19] Matsuoka T. Evaluation of monensin toxicity in the horse. J Am Vet Med Assoc 1976;169:1098–100.

[20] Ensley S, Mostrom M. Equine mycotoxins. Vet Clin North Am Equine Pract 2024;40:83–94. https://doi.org/10.1016/j.cveq.2023.10.002.

[21] EFSA, European Food Safety Authority. Opinion of the scientific panel on contaminants in the food chain [CONTAM] related to fumonisins as undesirable substances in animal feed. EFSA J 2005;235:16–7. https://doi.org/10.2903/j.efsa.2005.235.

[22] Khol-Parisini A, Hellweg E. Razzazi-Fazeli P, Saalmüller A, Strasser A, Tichy A, Zentek J. Highly deoxynivalenol contaminated oats and immune function in horses. Arch Anim Nutr 2012;66:149–61. https://doi.org/10.1080/1745039x.2012.672220.

[23] Malekinejad H, Maas-Bakker RF, Fink-Gremmels J. Bioactivation of zearalenone by porcine hepatic biotransformation. Vet Res 2005;36:799–810. https://doi.org/10.1051/vetres:2005034.

[24] Hartinger T, Kröger I, Neubauer V, Faas J, Doupovec B, Schatzmayr D, Zebeli Q. Zearalenone and its emerging metabolites promptly affect the rumen microbiota in Holstein cows fed a forage-rich diet. Toxins (Basel) 2023;15:185. https://doi.org/10.3390/toxins15030185.

[25] Vance CK, King EH, Bowers SD, Ryan PL, Walters K, Shappell NW. Reproductive performance of mares fed dietary zearalenone. Front Vet Sci 2019;6:423. https://doi.org/10.3389/fvets.2019.00423.

[26] Caloni F, Cortinovis C. Effects of fusariotoxins in the equine species. Vet J 2010;186:157–61. https://doi.org/10.1016/j.tvjl.2009.09.020.

[27] Kühnert M. Veterinärmedizinische Toxikologie: Allgemeine und Klinische Toxikologie; Grundlagen der Ökotoxikologie. 1. Jena Stuttgart: Fischer; 1991. p. 350–412. Aufl. ed.

[28] Plumlee KH. Pesticide toxicosis in the horse. Vet Clin North Am Equine Pract 2001;17:491–500. https://doi.org/10.1016/s0749-0739(17)30047-0.

[29] Kansas State Veterinary Diagnostic Laboratory, Diagnostic insights. https://ksvdl.org/resources/news/diagnostic_insights/june2023/poisonous-plants.html. (accessed 13 May 2025).

[30] Realini FM, Escobedo VM, Ueno AC, Bastías DA, Schardl CL, Biganzoli F, Gundel PE. Anti-herbivory defences delivered by Epichloë fungal endophytes: a quantitative review of alkaloid concentration variation among hosts and plant parts. Ann Bot 2024;133:509–20. https://doi.org/10.1093/aob/mcae014.

[31] Evert Ting WT, Chang C-H, Szonyi B, Gizachew D. Growth and aflatoxin B1, B2, G1, and G2 production by Aspergillus flavus and Aspergillus parasiticus on ground flax seeds (Linum usitatissimum). J Food Protect 2020;83:975–83. https://doi.org/10.4315/JFP-19-539.

[32] Wyse J, Latif S, Gurusinghe S, McCormick J, Weston LA, Stephen CP. Phytoestrogens: a review of their impacts on reproductive physiology and other effects upon grazing livestock. Animals 2022;12:2709. https://doi.org/10.3390/ani12192709.

[33] Nagy AL, Cortinovis C, Spicer LJ, Perego MC, Caloni F. Long-established and emerging pesticide poisoning in horses. Equine Vet Educ 2019;31:496–500. https://doi.org/10.1111/eve.12887.