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**The load of mycotoxins, phytoestrogens, pesticides and other toxins in a
broad range of feedstuffs and implications for horse health**

Diploma Thesis

University of Veterinary Medicine Vienna

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Vienna, January 2025

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Acknowledgements

I would like to express my deepest gratitude to all those who have contributed to the successful completion of this diploma thesis.

First and foremost, I would like to extend my sincere appreciation to my supervisor, Univ.-Prof. Dr.sc.agr. Qendrim Zebeli, for giving me the opportunity to work on this important and interesting project, for their invaluable guidance, continuous support, and constructive feedback throughout the research process. Their expertise and encouragement have played a crucial role in shaping this work.

I would like to acknowledge Dipl.-Ing.Dr.techn. Michael Sulyok for conducting the analysis with dedication and precision, as well as Vladimir Milojevic for their efforts in collecting the necessary samples for this research. Their contributions have been instrumental in the completion of this study.

I also extend my gratitude to the Members of the Clinical Department for Farm Animals and Safety of Food Systems, Centre for Animal Nutrition and Welfare for their support, insightful discussions, and assistance throughout the research process. Their contributions have significantly enhanced the quality of this work.

A special thanks goes to my family and friends for their unwavering support, patience, and encouragement. Their belief in my abilities has been a constant source of motivation throughout this journey.

I would also like to acknowledge my colleagues and fellow students who have provided valuable discussions, shared ideas, and offered moral support during this academic endeavor.

Lastly, I extend my appreciation to anyone who, directly or indirectly, contributed to the completion of this thesis. Your support and encouragement have been invaluable, and I am truly grateful.

Thank you all.

Abstract

This thesis investigates the prevalence of contaminants in horse feed and their potential implications for equine health. A total of 108 feed samples, including hay, grains, processed plant products, and supplementary feeds, were collected across Europe and analyzed for over 250 contaminants using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The study aimed to assess contamination levels, identify differences among feed types, and evaluate the potential health risks posed to horses.

Findings revealed that 99 % of the samples contained fungal metabolites, with *Fusarium* toxins being the most prevalent. High concentrations of phytoestrogens and plant toxins were observed, particularly in lucerne-based and mixed processed plant products, raising concerns about their potential cumulative health effects. Single feed products like hay exhibited lower contamination levels compared to supplementary feeds such as grain-free mashes and mueslis. Additionally, pesticide and veterinary drug residues were detected, with mash products showing the highest contamination load.

The study highlighted significant regulatory gaps, as current EU guidelines primarily focus on farm animals and inadequately address contaminants in horse feed. Hazardous substances like colchicine and monensin, which are toxic to horses, were identified in several samples, likely due to cross-contamination during production.

The thesis underscores the need for stricter regulations, routine testing, and targeted research to better understand the synergistic effects of co-contaminants. These measures are crucial for ensuring the safety of horse feed and safeguarding equine health.

Abstract

Diese Arbeit untersucht die Belastung von Pferdefutter mit Schadstoffen und deren potenzielle Auswirkungen auf die Gesundheit von Pferden. Insgesamt wurden 108 Futtermittelproben, darunter Heu, Getreide, verarbeitete Pflanzenprodukte und Ergänzungsfuttermittel, aus verschiedenen Ländern Europas entnommen und auf über 250 Schadstoffe mittels Flüssigchromatographie-Tandem-Massenspektrometrie (LC-MS/MS) analysiert. Ziel der Studie war es, die Kontaminationsniveaus zu bewerten, Unterschiede zwischen den Futtermittelarten zu identifizieren und die potenziellen Gesundheitsrisiken für Pferde einzuschätzen.

Die Ergebnisse zeigen, dass 99 % der Proben Pilzmetaboliten enthielten, wobei Fusarium-Toxine am häufigsten vorkamen. Hohe Konzentrationen an Phytoöstrogenen und Pflanzengiften wurden insbesondere in Luzerne-basierten und gemischten verarbeiteten Pflanzenprodukten festgestellt, was Bedenken hinsichtlich ihrer möglichen kumulativen Gesundheitseffekte aufwirft. Einzelfuttermittel wie Heu wiesen geringere Kontaminationsniveaus auf als Ergänzungsfuttermittel wie getreidefreie Mashies und Müslis. Zudem wurden Pestizid- und Tierarzneimittelrückstände nachgewiesen, wobei Mashies die höchste Belastung zeigten.

Die Studie hebt erhebliche regulatorische Lücken hervor, da die aktuellen EU-Richtlinien hauptsächlich auf Nutztiere abzielen und Kontaminanten in Pferdefutter unzureichend berücksichtigen. Gefährliche Substanzen wie Colchicin und Monensin, die für Pferde hochtoxisch sind, wurden in mehreren Proben gefunden, vermutlich durch Kreuzkontamination während der Produktion.

Die Arbeit betont die Notwendigkeit strengerer Vorschriften, routinemäßiger Tests und gezielter Forschung, um die synergistischen Effekte von Kontaminanten besser zu verstehen. Diese Maßnahmen sind entscheidend, um die Sicherheit von Pferdefutter zu gewährleisten und die Gesundheit von Pferden zu schützen.

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1. Introduction

As herbivores, the horse's diet consists mainly of roughage including grass from pasture or conserved grass products. The latter commonly include hay, grass silage, and haylage. The recommendations are to feed horses not less than 1.5 kg dry matter hay per 100 kg bodyweight (1). In addition to hay, various processed plant products can be found in the equine feed market both as single feed ingredients (i.e., hay cubes or lucerne cobs) and complementary feed (i.e., grain-free muesli or mash). Indeed, in the previous two decades the market for horse feed has constantly increased, offering a large variety of horse feed types. Besides common single feeds like grains, herbs, oilseed and byproducts, there are various supplementary feeds with or without feed additives on the market. Supplementary feeds and feed additives are taking an increasing role in equine nutrition, allowing more precise diet formulations to be made based on the specific requirements of horse types such as senior horses, breeding horses, foals and growing horses, and sport horses.

Besides being a source of nutrients for the horse, the feeds can also be a source of biotic agents that may contaminate them. This may include fungi, bacteria or their toxins, most importantly the mycotoxins produced by toxigenic fungi. In addition, there are several abiotic contaminants in the equine feeds such as plant toxins, phytoestrogens, pesticide and vet drug residues (2), which may pose a risk for horse health. The contamination of the horse feed can happen on the pasture or field, during harvesting, storage or during feed production as a cross contamination with other ingredients in the feed factories. This may increase the exposure of horses with direct implications for their health and welfare. Still, the feed regulation is mainly focused on farmed animals such as pigs, ruminants and poultry, which are used for food production. Horses are rarely within the category as scarcely used for meat and milk production, and are mainly considered as sport and leisure partners. General legal regulations like (EG) Nr. 767/2009 state that feedstuff should not pose risk to animal health, human health or the environment. The legal regulations are less stringent and often not classified for horses. Only one mycotoxin, a toxin produced by fusarium species, has guidance levels for horse feed, which is fumonisin B1+B2.

This study aimed at investigating whether the presence and concentration of contaminants in horse feeds is significantly associated with the type of product and specific ingredients. Moreover, it was hypothesised that due to lacking legal regulations horse feeding products

might contain substances in concentrations that might be potentially hazardous to a horse's health.

Given the various ways horse feed can become contaminated, it is notable that, to the best of our knowledge, only one study has dealt with that topic and focused exclusively on mycotoxins. The study of Liesner et al. (3) analyzed 62 complementary horse feeds available on the German market, most of which contained high levels of grains. The findings revealed that the products exhibited co-contaminations, but all detected mycotoxins were present only at low levels (3). To our knowledge, no other study has examined a broader range of contaminants or focused on a wider variety of horse feeds. Furthermore, the impact of most potential contaminants on horse health has yet to be thoroughly investigated, in order that recommendations and regulations can be proposed.

In the following chapter, the thesis introduces the most important facts about horse feeding and its legal regulation while comparing it to the existing literature. In the third chapter, the samples investigated and analysing methods are shown in detail. Afterwards, the results of the analyses are shown, and in the final chapter the findings are discussed. In the end, the main findings are briefly summarized.

2. Literature Review

2.1. Introductory remarks to horse feeding

As herbivores, the horse's feeding is primarily based on roughage, which provides mainly energy, protein, and most importantly structural fibres. Roughages such as pasture grasses or conserved roughages in form of hay, haylage or silage usually provide enough energy and nutrients for horses especially when kept at maintenance level without work (1). However, roughages differ in their quality and availability, and they normally are low in certain nutrients such as certain amino acids, fatty acids, minerals, and trace elements. Therefore, they may require supplementation to fulfil their needs for these nutrients (1). Other categories of horses such as sport horses, breeding horses, foals or seniors typically require supplementation of the roughage diet, as the forage is unable to meet their nutritional demands with energy, protein and other nutrients. This is commonly achieved by feeding horses cereal grains, tubers or oilseeds as energy source, and legume seeds or oilseed products as protein and lipid sources. In addition, concentrate mixtures are also offered as a source of energy/protein or minerals/vitamins, mainly as commercially available as muesli, pellets or other confectionary forms. Undernutrition is for horse harmful as overnutrition. It is estimated that over 40 % of the horse population in various countries is overweight (4). This can lead to metabolic issues such as Equine Metabolic Syndrome (EMS) and laminitis, as well as reproductive problems (5).

2.2. Feeds for horses

In this section, we will describe the various types of feeds that are essential to maintaining the health and performance of horses. In terms of the ingredients used, the equine feeds are categorized as single feedstuffs and the supplementary feedstuffs. The first includes single ingredients more commonly for horses such as roughages, tubers, and various single concentrate courses. Supplementary feedstuffs consist in mixtures containing more than one component. The roughages, such as hay and pasture, form the foundation of a horse's diet and are crucial for proper digestion and gut health. Further single feedstuffs as well as supplementary feed, e.g. muesli and mineral feeds, added to the daily diet offer additional energy and nutrients, particularly for horses with higher nutritional requirements, such as those in heavy work or breeding.

2.2.1. Roughages and hay replacement products for horses

Pasturing is the ideal form of horse feeding both in terms of nutrition and welfare. Yet, not every vegetation stage and type of pastures is suitable to horses. In addition, the pasture grass is only available during few months. Therefore, the grass is commonly conserved, mainly as hay, haylage, and silage, which are key components of a horse's diet. Hay, unlike haylage or silage, is normally harvested later and requires a longer drying period. Therefore, horse hay typically contains more crude fiber but less crude protein and minerals. The key advantage of ensiled roughage, such as haylage or silage, lies in its higher nutritional concentration, which is a result of the earlier harvest and shorter drying period. This shorter drying time also reduces the dependency on favorable weather conditions for producers. The preservation process for silage and haylage occurs under oxygen-free conditions, leading to the production of organic acids. Additionally, the higher moisture content in silage and haylage results in lower dust levels, making them more suitable for horses with respiratory issues. The recommendations are to feed horses a minimum of 1.5 kg of hay per 100 kg of bodyweight per day (1). Due to the anatomical and physiological characteristics of horses, such as their teeth with characteristic enamel ridges and large intestines, such as colon and caecum, a high fibre content is necessary to keep their digestive system functioning properly. The structure stimulates enough chewing activity of the horse and leads to higher saliva production. Saliva contains bicarbonate which works as buffer for stomach acid.

The fibrous structure of these feed materials also ensures that horses have long eating periods, which is crucial for their well-being. In the wild, horses spend up to 16 hours a day feeding, so it is important that domesticated horses also have extended feeding times to meet their needs. Otherwise, it could lead to problematic behavior and Equine Gastric Ulcer Syndrome (EGUS) and other health issues. Older horses, however, lack in ability to break down hay due to deteriorating dental structure and reduced chewing muscle strength. Moreover, colic, such as impaction, may occur when horses do not chew hay thoroughly, causing large pieces to be ingested. For these horses, processed hay products can effectively replace hay in their diet. In addition, these products require less space and can be stored and transported much easily to be used as hay replacement in case it is not available. These products commonly called cubes, pellets or briquettes made of meadow hay or other plants such as lucerne or green oat. These products consist of chopped hay or the other plants, harvested at an early vegetation phase, dried mainly indoors, and produced under the use of heat. Due to early harvest, indoor drying and heat processing, these products have higher nutrient composition, and maintain both the

green color and the main product odor, having an advantageous nutritive and hygienic value. However, these products contain less crude fiber and have little structural value. Also, most of these products need to be soaked in water before feeding to prevent pharyngeal congestion as they expand in volume. While some products, like flakes, can be fed dry, it is generally safer to soak them all. Consequently, these feedstuffs contain less structure, and horses will be less stimulated to chew and will eat their feed faster. Nevertheless, processed plant products still provide the necessary nutritional balance that horses would get from traditional roughages and are a good alternative for older horses or those with dental issues. Clover and lucerne are legumes and can also be conserved as hay or processed hay. These types of roughage contain higher levels of protein.

Depending on the vegetation stage and the region where the grass was conserved the nutritional composition varies. Each region has different mineral profiles due to the soil. It is important to know nutritional content of the roughage to accurately calculate the additional diet of a horse. Therefore, analyses of the roughage can be useful.

2.2.2. Grains, legumes, oilseeds, tubers, and their products

Horses with higher nutritional needs beyond basic maintenance requirements require additional feeds with high content in energy and nutrients. Grains, due to their high starch content, can provide extra energy in a horse's diet. Although excessive starch in a horse's diet can lead to digestive disturbances like colic and laminitis, as the horse's hindgut bacteria rapidly ferment undigested starch, producing harmful acids and toxins (6). Nearly all grains need to be pretreated to breakdown the endosperm and make the starch more digestible. Depending on the type of grains, they are crushed, rolled or heat-treated. From grains, oats are generally considered more suitable for equine diets due to their higher fiber content and ease of prececal digestion of its starch. They are also the only grain which normally does not necessary need pretreatment. Other grains typically fed to horses are barley and corn, whereas wheat, rye and triticale are less used in horse feeding due to their greater risk for stomach digestive upset and metabolic disorders in horses. To increase the digestibility of starch, grains such as barley and corn, but also oats are commonly processed/flaked. Several grain-flaked products are commonly found on the equine feed market, offered both as single or part of supplementary feeds or muesli.

Legumes are rich in proteins and, consequently, amino acids, making them important for muscle development in sport horses and for breeding horses. Commonly used legumes are

soybeans, beans, and their products. In horse feeding, tubers like carrots and sugar beet pulp provide digestible energy with lower starch content, reducing the risk of digestive issues. Sugar beet pulp is a product of sugar beets after extractions of sugar/molasses and is commonly fed to horses as sugar beet pulp is almost as rich in energy as oat, while its main energy source is fermentable fiber like pectin instead of starch. Because of high pectin amounts, this product helps maintain hydration because it absorbs water when soaked, forming a moist mash that provides additional water intake. Carrots also offer essential vitamins like beta-carotins which are converted to vitamin A, making these tubers a good supplement especially for young, breeding and older horses. Carrots are also offered dry as part of the supplementary feed/muesli. Oilseeds, including flaxseed and sunflower seeds, offer a concentrated source of energy in the form of long chain fatty acids, reducing the reliance on high-starch feeds and thereby mitigating the risk of digestive disturbances in horses. Rich in alpha-linolenic acid (omega-3 fatty acids), flaxseed can contribute to enhanced coat condition and anti-inflammatory effects (7,8). In addition, some oilseeds also contain other beneficial ingredients, such as flaxseed hold mucilage. To achieve a balanced diet for horses, roughage can be supplemented with grains, legumes, and their byproducts.

2.2.3. Supplementary feeds

The previously described single ingredients can be fed separately or together to horses. When two or more single ingredients – that may include or not minerals and feed additives- are mixed, they are legally considered as mixed or compound feeds. In horses, the compound feeds typically used are the supplementary feeds, which are used to complement the equine diet with the lacking energy and nutrients, mainly protein, minerals, or vitamins. Several types of equine supplementary feeds are commercially available such as energy, protein, and mineral-vitamin supplementary feed for different horse groups. Depending on the type, they may contain products of grains, legumes, oilseeds, tubers, minerals, vitamins and other nutritional, zootechnical or technological feed additives.

Nowadays, the equine supplementary feed products are mainly produced as muesli and mashes, and less as ground or pelleted form. Per definition, supplementary feeds help to prevent deficiencies in energy or essential nutrients like amino acids, fatty acids, minerals, trace elements, and vitamins. There are a large range of mueslis on the market often containing grain products (rolled, flaked, puffed), legumes like soybeans, oilseeds or their by-products and mineral-vitamin pellets. The composition of compound feeds varies considerably and their inclusion in the diets depends specifically on the intended specific horse

requirements. These feed mixes can also be pelletized, which has the advantage that horses are not able to select only certain ingredients which they like while also preventing the segregation of the ingredients though it is rather common for cattle and pigs. There are specific mueslis for breeding mares or foals. For example, a lactating mare requires more energy and protein and should, in addition to roughage and oats, be fed muesli or pellets with high concentrations of essential amino acids, fatty acids and vitamins and minerals (1). Racehorses, on the other hand, need higher energy in the form of carbohydrates and protein. They also have a higher demand for electrolytes due to the significant loss through sweating as well as for vitamins and minerals, such as Vitamin E, which has antioxidant effects on cells.

Mash is another form of a supplementary feed which needs to be soaked before feeding. Therefore, its high moisture content promotes hydration, which is particularly important during colder months when horses may be less inclined to drink water. Additionally, mash is highly palatable, making it an effective option for encouraging consumption, e.g. horses recovering from illness. The soft texture facilitates easier chewing and digestion for older horses or those with dental issues, thereby reducing the risk of choke. Such as muesli, mash can be customized with various ingredients and feed additives, allowing for tailored nutrition to address specific dietary needs and health conditions.

During the last years, several brands of grain-free muesli and mashes are available on market. These feeds, by definition, do not contain starchy grains and their products, mainly for dietetic reasons. For example, horses with metabolic diseases or those prone to obesity, grain-free options are a good choice. Grain-free feeds typically consist of fiber-based forage products (e.g. hay, alfalfa or straw chops or cobs), alternative fibre and fat-rich energy sources like brans, beet pulp, soy hulls or oilseeds. Due to their lower concentration of starch, they can be used for horses with metabolic issues like Cushing and are especially a good alternative for older horses to feed them more nutrients without the high starch concentration of grain-based muesli.

Additionally, to the above-mentioned supplementary feeds, in the equine market there are multiple brands of supplementary mineral feeds available. These supplementary mineral feeds consist of different sources of mineral, trace elements and vitamins with or without other feed additives to complement the equine diet with essential minerals and vitamins or other active substances. To increase palatability or stability, or to modify texture, these products may

contain also other ingredients such as grain brans, molasses, preservatives, stabilisators, antioxidants, or taste modifiers, and they can be pulverized or pelletized.

During the last years, several nutritional supplements have emerged in the equine market, which are commonly used to complement the diet with nutrients (minerals, vitamins) and also active substances to support various organs (i.e., skin, joints etc.). Besides Methyl Sulfonyl Methan (MSM) to support joints and the musculoskeletal system, dried herbs and herbal extracts are also being used, either separately or as part of the supplementary feed for different reasons such as to improve the palatability or stability of the feed, add minerals, provitamins and phytochemicals to the diet and for their phytochemical benefits. As an example hawthorn (e.g. "Weißdorn") is helpful for cardiac issues and willow bark (e.g. "Lexa Weidenrinde") can be used in feverish conditions due to its antiphlogistic effects (9).

2.3. Potential feed contaminants and their consequences for equine health

During their production, processing, storage and transport, horse feeds may be contaminated with harmful substances for their health or the health of humans and the environment. Therefore, production and commercialization of the feed including the equine feed is legally regulated. Next to further national laws, animal feed production is mainly regulated by the EU. Producers require authorization or official registration, and controls are executed by local authorities. Thus, it must comply with Regulation (EC) No. 767/2009 regarding quality and labelling and must not pose a risk to animal or human health or the environment. Feed additives and supplementary feeds containing them must comply with EU Regulation (EC) No. 1831/2003. Additionally, there is a list of prohibited substances, including feces, packaging, and waste. Feed is also regulated by the directive 2002/32/EC, a list of unwanted substances, such as arsenic, aflatoxin B1, and theobromine, each with a specified maximum concentration. These legal regulations not only protect animals but also aim to minimize the risk to humans who consume animal products. However, it is important to note that while legal restrictions exist, they do not apply to all toxins and do not prevent small quantities from being found in horse feed. Even small amounts of mycotoxins and other contaminants can lead to health consequences.

2.3.1. Biotic contaminants

Biotic contaminants in horse feed refer to metabolites produced by living organisms such as insects and microorganisms (e.g. bacteria and fungi) that can compromise the health of horses.

These contaminants can lead to various diseases, nutrient deficiencies, and other health issues. Below the contaminants associated to selected microorganisms are described.

2.3.1.1. Fungal & endophyte toxins

Mycotoxins are secondary metabolites produced by certain toxigenic fungi, commonly categorized into field and storage fungi depending on the infestation of mycotoxins during or after harvest, respectively (1). Conditions of high humidity and temperature enhance growth for both types (10). On the other hand, Son et al. (2) found a correlation between dry weather conditions and the load of mycotoxins in horse pasture. These compounds commonly contaminate crops and animal feed, potentially posing significant health risks to both animals and humans. Produced mainly by fungi such as *Aspergillus*, *Fusarium*, and *Penicillium*, mycotoxins are a major concern for food safety. Mycotoxins are the most significant group of toxins in animal feed. However, only five mycotoxins are regulated under EU guidelines, specifically Directive 2002/32/EC and Recommendation 2016/1319. These include aflatoxin B1, ergot alkaloids, and mycotoxins produced by *Fusarium* fungi. Aflatoxins, which are produced by *Aspergillus* fungi, are classified as Group 1 human carcinogens by the International Agency for Research on Cancer (IARC). They have also been shown to have hepatocarcinogenic effects in animals (11). Braga et al. investigated the effects of mycotoxins on horses during short-term exercises while demonstrating that the presence of aflatoxin B1 (AFB1) in horse diets significantly influenced alkaline phosphatase activity, suggesting a hepatotoxic impact (12). Ergot alkaloids are a group of naturally occurring compounds produced by certain fungi, particularly the *Claviceps* species as well as *Neotyphodium* species, posing a risk for both livestock and human (13). Ergot alkaloids can impair reproductive ability in horses (14). Although their high toxicity in other animals suggests that horses might also exhibit additional symptoms, there are few documented cases in horses (13).

Studies indicate that 50 % of farm animal feed samples are contaminated with low levels of *Fusarium* toxins (15). Research by Son et al. suggested a correlation between dry weather conditions and the presence of mycotoxins on horse pastures used for grazing or in conserved roughage (2). Mycotoxins produced by *Fusarium* fungi are divided into two groups: guidance value mycotoxins and emerging mycotoxins. A study by Liesener et al. (3) investigated the mycotoxin load in 62 German horse complementary feed (single and compound feed) samples, all of which contained high amounts of cereals. Nearly all samples were tested positive for guidance-level *Fusarium* toxins, though generally at low concentrations. There are

legal regulations governing the permissible levels of these mycotoxins in feed. The only guidance value mycotoxin that currently has EU regulations for horses is fumonisin B1+B2. Intoxication with this mycotoxin can cause equine leukoencephalomalacia (16). In horse feed, a concentration of up to 5 mg/kg in feed with 12 % moisture content is allowed, concerning the EU recommendation 2016/1319.

Deoxynivalenol (DON) is known to be frequently present in low concentrations (17). Although there are no legal regulations for DON in horse feed, its intake has significant impacts on pigs and ruminants, while studies suggest it has a lesser impact on horses (18). However, there is evidence of synergistic effects when DON is present alongside other *Fusarium* toxins, which can lead to weight loss due to decreased feed intake (19). For this reason, it may be prudent to adapt the allowable levels for swine feed (0.9 mg/kg) and ruminant feed (2 mg/kg) for horses as well. Zearalenone, another mycotoxin, has hyperestrogenic effects. However, its overall toxicity is relatively low (20) although Songsermsakul et al. investigated the metabolism of Zearalenone and its metabolites in horses fed *Fusarium*-contaminated oats (21). They demonstrated that horses primarily convert Zearalenone into β -zearalenol (β -ZOL), which contrasts with other species like swine, where α -zearalenol (α -ZOL) is the predominant metabolite. This metabolic pathway may explain why horses exhibit a different sensitivity to Zearalenone compared to other animals, such as pigs, which are more susceptible to its estrogenic effects. The study by Songsermsakul et al. also highlighted the extensive glucuronidation of Zearalenone and its metabolites in horses, with nearly complete conjugation in plasma and urine (21). This suggests an efficient detoxification process that could mitigate the potential adverse effects of Zearalenone exposure. However, despite this detoxification, the presence of Zearalenone and its metabolites in various biological matrices indicates a continuous exposure risk. Schumann et al. explored the immunotoxic effects of DON and Zearalenone on equine peripheral blood mononuclear cells (PBMC) by demonstrating that DON and Zearalenone, along with their metabolites, could significantly reduce the viability and proliferation of equine immune cells in vitro (22). This highlights the potential for these mycotoxins to compromise immune function, even at low exposure levels. In addition to that, the authors found that the IC₅₀ values for DON and its acetylated form were lower than those for Zearalenone and its metabolites, indicating a higher cytotoxicity of DON to equine immune cells (22). The study by Dänicke et al. also highlighted the role of mycotoxins in exacerbating intestinal inflammation and compromising the mucosal barrier, which could facilitate the transfer of toxins into systemic circulation (23). This mechanism may explain the higher

incidence of DON in colic-affected horses, as intestinal lesions could enhance toxin absorption. The study's association between DON exposure and increased activities of liver-related enzymes, such as aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT), indicates possible liver involvement in mycotoxin metabolism and its role in systemic inflammation.

2.3.1.2. Bacterial toxins

It is quite unusual to find bacterial toxins in feed that is not contaminated. However, the study of Son et al. (2) found also that one horse pasture used for grazing and the production of hay was contaminated with cereulide, which is a toxin produced by *Bacillus cereus*. According to Steiner et al. (24), bacterial metabolites such as monactin, nonactin, and the aflatoxin B1 precursor averufanin were found as contaminants in cattle and chicken feed. The macrotetrolide antibiotics monactin and nonactin were also detected in wheat and corn silage in a study by Shimshoni et al. (25) in wheat and corn silage. While the effects on ruminants are not clear those on horses are even less understood.

2.3.2. Abiotic contaminants

Abiotic contaminants in horse feed refer to non-living substances that can enter the feed during production, processing, or storage, potentially posing health risks to horses. These contaminants include plant toxins and metabolites, pesticides, and veterinary drug residues that can be introduced through contaminated soil, water, or air, as well as through the use of certain agricultural practices. There are several potential abiotic contaminants in feeds, but this chapter deals with plant toxins, veterinary drugs and pesticides. Plant toxins, such as colchicine produced by meadow saffron (*Colchicum autumnale*), are known to be highly toxic to horses. This plant is commonly found on extensively used pastures, and its toxicity persists even after the plant is dried during the conservation process. The toxicity of colchicine is due to its cytotoxic effects. An intake of 1200–3000 grams of fresh plant material per horse can be fatal, or as little as 1 mg of colchicine per kg of body weight whereas a dose as low as a quarter of this amount can already lead to diarrhea (26,27). Typical symptoms of colchicine poisoning include colic, hemorrhagic diarrhea, and circulatory failure. Although there are reports suggesting that horses may reject *C. autumnale* in hay (28), a study by Mueller et al. (29) found that horses can ingest both leaves and capsules present in hay.

Another significant concern are cyanogenic glycosides, like linamarin which occurs in flaxseeds (1). This cyanogenic glycoside can release hydrogen cyanide when metabolized, a

potent toxin that can hinder the transport of oxygen through the blood. While flaxseeds contain about 0.2 mg of hydrogen cyanide per gram, a horse would need to consume several kilograms to reach a toxic dose. Daily amounts of up to 120 g are considered safe for adult horses, even when fed dry. White clover can also contain high concentrations of glycosides such as linamarin and lotaustralin, posing a significant concern for grazing animals (30). Ingestion of cyanogenic glycosides from white clover might be associated with equine grass sickness (EGS), although the exact relationship is unclear, particularly concerning the timing of exposure and onset of illness (31). Certain pesticide, biocide, and veterinary drug residues found in the environment can have effects on mammals and other species.

Phytoestrogens, like Biochanin, Coumestrol and Daidzin are naturally occurring plant compounds that structurally resemble 17β -estradiol and can mimic or modulate its activity in the body. These compounds are produced by a variety of plants, especially alfalfa, clover and other legumes. Phytoestrogens are synthesized by plants as secondary metabolites, often in response to environmental stressors such as UV radiation or pathogen attacks. Due its similarity to 17β -estradiol it can bind on the receptors and can have influence on the oestrous cycle. It is known to lead to reproductive issues in sheep, cattle and horses (32). The study of Son et al.(2) showed that not only the plant composition of a pasture, like a high number of legumes, leads to high concentrations of phytoestrogens, also other factors must be relevant, although these factors are yet to be determined.

2.3.2.1. Pesticides residues

Designed to control pests, these chemicals can inadvertently impact non-target species, including mammals. Piperonyl butoxide, a synergist used in many insecticides, was found in 39% of dairy cow feed samples in Austria (33). Animal studies have shown that exposure to piperonyl butoxide can cause foetal malformations and abnormalities (34). Furthermore, Horton et al. found that exposure to piperonyl butoxide was negatively associated with neurodevelopment in humans (35).

Didecylmethylammonium chloride (DDAC) is a biocide and disinfectant used as a preservative in household products. DDAC can cause pulmonary toxicity and cytotoxicity in human alveolar cells (36). Tebuconazole, a fungicide, is known to have endocrine-disrupting effects on fish and other species (37).

2.3.2.2. Veterinary drugs residues

Drugs administered to livestock and pets can persist in the environment through manure and other waste products. These residues can contaminate feed and water sources, affecting non-target animals. Monensin, an ionophore antibiotic used as an anticoccidial agent for ruminants and poultry, is highly toxic to horses, with a median lethal dose (LD50) of 2–3 mg/kg of body weight (38). According to Commission Directive 2009/8/EC, up to 1.25 mg/kg of monensin is allowed in feed with a moisture content of 12 %. Typical symptoms of monensin toxicosis in horses include anorexia, ataxia, and sweating (38). Dinitrocarbanilide, the chemical name for Nicarbazin, is a coccidiostat used for poultry. An allowable concentration of 0.5 mg/kg of Nicarbazin is set in the Directive 2009/8/EC for equine feed with a moisture content of 12 %. The toxicity of Nicarbazin is not fully evaluated, but it may have fetotoxic effects (39).

3. Material and methods

3.1. Feed sampling

For this study, 108 single and supplementary horse feeds available on the equine market were randomly collected from ready-to-feed products of various local commercial providers in Germany, Austria, France, Switzerland, and Hungary. 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 at 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 ≤ 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 (± 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 mycotoxins and other fungal secondary metabolites.

Table A provides information of the feeds tested with names, brand name, type and producer/provider. The tested single feeds included:

- 1) roughages such as hay (n = 5),
- 2) processed plant products (n = 24) like hay cubes, lucerne cubes or gras and lucerne mix cobs or flakes,
- 3) grains (n = 7) such as oats, maize flakes, and barley flakes,
- 4) herbs (n = 6) consisting of willow bark, hawthorn leaves and blossoms and various herb mixes.

The supplementary feed samples included:

- 1) grain-based muesli (n = 24) for seniors, breeding horses, Icelandic horses and as oat replacement,
- 2) grain-based mash (n = 5),
- grain-free muesli (n = 12)

grain-free mash (n = 8),

mineral feed (n = 5).

Other single feed samples were also collected such as byproducts sugar beet pulp (n = 7), wheat bran (n = 2), soybean meal (n = 1), oilseeds such as linseeds (n = 1) and treats made of bread (n = 1).

Based on their composition and feeding goal, the products were categorized into hay, processed plant products, grains, herbs, grain-based and grain-free mash, grain-based and grain-free, byproducts and others.

3.2. Feed analyses

The horse feed samples underwent testing to detect over 250 different contaminants, including biotoxins, pesticides, and veterinary drugs. 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 specialized equipment capable of identifying a broad spectrum of substances (24). Subsequently, 83 of these products were further examined for pesticide and veterinary drug residues. Additionally, official ingredient information was incorporated into the analysis.

3.3. Statistical analyses

For the statistical analysis, the horse feeding products were grouped as follows: hay (n=5), processed plant products (n=24), grains (n=7), muesli (n=24), grain-free muesli (n=12), mash (n=5), grain-free mash (n=8), herbs (n=6), byproducts (n=11), and others (n=6). The "others" category primarily included mineral feed and treats. Contaminants were classified into 15 groups, including mycotoxins, plant toxins, phytoestrogens, bacterial metabolites, lichen metabolites, plant metabolites, and unspecific metabolites. The mycotoxin group comprised *Alternaria* metabolites, *Aspergillus* metabolites, *Fusarium* metabolites, *Penicillium* metabolites, ergot alkaloids, and other fungal metabolites.

The statistical analysis involved descriptive statistics and analysis of variance (ANOVA). Global F-tests were conducted to identify significant relationships, and the Tukey-Kramer test was used to analyze significant differences among horse feeding products within the mycotoxin groups.

4. Results

4.1. Overall occurrence and concentrations of contaminants in the feed samples

Table 1 shows that eleven 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. Additionally, 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. Veterinary drug residues were present in 20.5 % of the 83 samples that were specifically tested for pesticides and veterinary drug residues. Ergot alkaloids and lichen metabolites were also found at lower rates, 62 % and 57 % respectively. The highest mean concentrations were observed in the groups of phytoestrogens (35 mg/kg) and plant toxins (38 mg/kg). The lowest mean concentration was detected for bacterial metabolites, at just 0.6 µg/kg. The only sample without any contamination was a product consisting purely of methylsulfonylmethane.

Table 1: Occurrence and concentrations of the contaminants in the analyzed equine single and supplementary feeds

Groups	No. of samples	No. Positive	% positive	Concentration ($\mu\text{g}/\text{kg}$)			
				Mean	SD	Maximum	Median
Total fungal metabolites	108	107	99	3,030.2	3,295.3	19,224.8	2,095.2
Alternaria metabolites	108	106	98	1,055.9	1,745.3	13,574.0	610.0
Aspergillus metabolites	108	100	93	290.5	917.8	8,758.3	56.4
Fusarium metabolites	108	107	99	1,003.2	1,020.1	6,174.2	821.0
Penicillium metabolites	108	106	98	421.5	1,120.5	8,221.3	143.1
Ergot alkaloids	108	67	62	63.0	122.3	898.5	10.4
Other fungal metabolites	108	106	98	196.0	349.3	2,826.5	75.9
Bacterial metabolites	108	17	16	0.6	1.9	12.7	0.0
Lichen metabolites	108	62	57	37.6	149.5	1288.5	1.6
Phytoestrogens	108	104	96	35,055.9	60,754.6	54,7595.6	23,337.5
Plant metabolites	108	97	90	1,021.4	1,202.4	5,738.4	682.7
Plant toxins	108	101	94	38,268.6	51,983.2	20,5624.4	10,034.4
Unspecific metabolites	108	107	99	1,762.5	2,222.3	16,262.4	1,192.8
Total pesticide residues	83	82	99	157.6	191.7	1,059.6	100.0
Total veterinary drug residues	83	17	20.5	122.3	558.8	4,223.45	0.0

4.2. Number of contaminants and co-contaminations in the feed samples

When examining the number of metabolites found in each sample (Figure 1), Fusarium metabolites were detected most frequently across all types of horse feeds. Other groups of

contaminants that were commonly observed across all sample groups included other fungal metabolites and unspecific metabolites. Single feed products like hay, grains, and byproducts were found to contain fewer metabolites compared to supplementary feed.

Each product group exhibited complex co-contaminations. Across all types of feed, *Fusarium* metabolites were the most prevalent group of metabolites. Unspecific metabolites and other fungal metabolites were also more common than other metabolite groups. The least frequent metabolites were bacterial and plant metabolites. Processed plant products displayed a more complex fungal metabolite profile than single feed items.

4.3. Differences of contaminations between the product groups

An important question is whether the concentrations of contaminants differ significantly across the various groups of horse feeds. Table 3 shows that *Aspergillus* (1.9 mg/kg) and *Penicillium* (2.1 mg/kg) metabolites were detected at the highest concentrations in mash. Plant toxins were found at the highest concentration of 96.4 mg/kg in grain-free mash. Other fungal metabolites had the highest concentration of 0.9 mg/kg in hay. In general, single feed products had a lower contaminant load. The concentrations of mycotoxins produced by *Fusarium* fungi, specifically those mycotoxins categorized by guidance values, were significantly higher in herbs. For example, the concentration of deoxynivalenol was significantly higher in herb products, at 0.99 mg/kg, compared to other feed groups. Other *Fusarium*-produced mycotoxins showed no significant differences between the horse feeds.

4.3.1. Contamination load in different roughage groups

Significant differences in contamination were observed among different types of roughage. Table 4 shows that processed plant products have higher concentrations of phytoestrogens, plant metabolites, and plant toxins compared to hay. While ergot alkaloids were not found in hay, they were detected at the highest concentration of 0.1 mg/kg in grass-based processed plant products. In Lucerne-based plant products, the concentration of ergot alkaloids was significantly lower, at just 0.03 mg/kg. 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-based (49.1 mg/kg) and mixed-based plant products (60.9 mg/kg) than in hay and grass-based plant products. Plant toxins were also observed at the highest concentration of 113.6 mg/kg in mixed-based processed plant products. The

number of metabolites detected was significantly higher in grass-based products for nearly all mycotoxin groups, unspecific metabolites, and phytoestrogens. Bacterial metabolites and plant toxins were more frequent in mixed plant products. When comparing the number of metabolites detected with their concentrations, it is evident that other fungal metabolites were more often found in grass-based products ($n = 14.8$) than in hay ($n = 8.8$), but they reached the highest concentration of 0.9 mg/kg in hay. Processed plant products exhibited higher concentrations in 40 % of the contaminants and a higher number of contaminants in 75 % of the contaminant groups.

4.4. Occurrences of abiotic contaminants

The total load of pesticide residues was higher in supplementary feed than in processed plant products (0.09 mg/kg) and grains (0.08 mg/kg), reaching its peak at 0.3 mg/kg in mash. There were no significant differences between the feed product groups regarding the concentration of veterinary drug residues. The concentration of piperonyl butoxide was higher in products containing grains and was significantly elevated in cereals, measuring 0.1 mg/kg. The occurrence of insecticide residues ($n = 3.4$) and fungicide residues ($n = 7.4$) was most frequent in mash, resulting in a significantly higher total number of pesticides ($n = 10.8$) in mash. Single feed products again exhibited a lower load of pesticide residues compared to supplementary feed. Fungicide residues were the most frequent type of pesticide residues detected. Piperonyl butoxide, tebuconazole, and didecyldimethylammonium chloride were the most frequently found pesticides, while monensin and dinitrocarbanilide were the most commonly detected veterinary drug residues.

In this study 96 % of the samples were detected with phytoestrogens, 90 % with plant metabolites and 94 % with plant toxins. They also showed the highest mean concentration with phytoestrogens at 35 mg/kg and plant toxins at 38 mg/kg. Phytoestrogens showed the highest concentration in grains, although there was no significant difference between the product groups. While comparing different types of roughage we found that phytoestrogens were found in significantly higher concentrations in lucerne based and mixed based processed products than in hay and grass based processed plant products. Although the number of metabolites detected in these products was significantly higher in grass based products, but obviously in lower concentrations. Plant metabolites were found in the highest concentration in processed plant products and significantly lower in grains and byproducts. The highest concentration of plant toxins was detected in grain-free mash.

4.5. Comparison of results with those from the literature

Fumonisin B1 + B2 is the only *Fusarium* toxin with an EU-regulated guidance value found in the EU Recommendation 2016/1319. In horse feed, a concentration of up to 5 mg/kg in feed with 12 % moisture content is allowed. The highest concentration we found was 0.052 mg/kg in a cereal product.

Deoxynivalenol (DON) does not have a maximum allowable concentration for horses. However, the highest concentration permitted for swine feed is 0.9 mg/kg, and for ruminants, it is 2 mg/kg. The highest DON concentration we found was 2.2 mg/kg in a processed plant product. Although studies indicate that DON has a greater impact on the health of pigs and ruminants than on horses, it would be prudent to adapt these established guidance levels for horse feed products, as the effects of co-contaminations have not yet been fully evaluated.

Colchicine is a toxin found in the plant meadow saffron (*Colchicum autumnale*). A dose of 1 mg of colchicine per kg of body weight can be lethal for a horse (3,4). It is known that feeding hay contaminated with *C. autumnale* poses a risk of intoxication. 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 in our study was 29.6 mg/kg in a mash from Hungary, with a feeding instruction of 50–200 g per 100 kg of body weight. The second-highest concentration detected was 8.4 mg/kg in a processed plant product from Hungary, which should be fed at a rate of 1000–1500 g per 100 kg of body weight. Another notably high concentration was 4.7 mg/kg in a grain-free cereal product from Hungary, which can be fed to a horse at 200–400 g per 100 kg of body weight. All other samples contained colchicine at concentrations below 0.05 mg/kg of feed.

Monensin is an anticoccidial drug used for ruminants and poultry. Horses are much more sensitive to this drug, with a median lethal dose of just 2–3 mg/kg of body weight (38). The legal regulation (EC/2009/8) allows up to 1.25 mg/kg of monensin in feed with a moisture content of 12 %. The highest detected concentration in this study was 1.9 mg/kg in a grain-free cereal product from Hungary. Horses can be fed 200–400 g per 100 kg of body weight. Two other products from Hungary contained 0.2 mg/kg of monensin: one was a cereal product, with feeding instructions of 400–1200 g per 100 kg of body weight, and the other was a mash, with feeding instructions to feed 50–200 g per 100 kg of body weight. Monensin is authorised as a feed additive for poultry but not for horses. The most likely explanation for contamination

is that residues of monensin are transferred from poultry feed to the subsequent horse feed produced during the manufacturing process.

Table 2: Concentration of 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)	Processed plant products ¹ (n=24)	Grains (n=7)	Herbs (n=6)	Mash (n=5)	GF-Mash ² (n=8)	Muesli (n=24)	GF-Muesli ² (n=12)	Byproducts (n=11)	Others ³ (n=6)	
Alternaria metabolites	1281.6	1400.1	502.9	1261.7	1325.2	589.3	1331.2	813.8	577.1	588.8	0.880
Aspergillus metabolites	735.8 ^{ab}	187 ^b	19.3 ^b	167.5 ^b	1889.2^a	411.6 ^(b)	62.6 ^b	289.7 ^b	129.3 ^b	488.6 ^{ab}	0.015
Fusarium metabolites	1301.1	1154.1	568.9	1406.4	1803.4	823.3	949.8	962.2	575.2	908.5	0.489
Penicillium metabolites	64.1 ^(b)	716.2 ^{ab}	20.8 ^b	627.7 ^{ab}	2123.7^a	256 ^(b)	135.7 ^b	243.5 ^b	162.6 ^b	578.4 ^{ab}	0.033
Ergot alkaloids	0	62.6	0	0	4.1	115.5	76.7	170.0	21.4	40.4	0.024
Other fungal metabolites	899.9^a	249.4 ^b	9.4 ^b	324.7 ^(b)	236.2 ^b	234.6 ^b	102.5 ^b	215.4 ^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	45.2 ^b	0 ^b	272.7^a	22.1 ^{ab}	9 ^b	35.6 ^b	14.4 ^b	0 ^b	3.4	0.04
Phytoestrogens	5347	44882	857348	21645	2371	27412	37420	40796	53472	57449	0.548
Plant metabolites	462.4 ^b	2196.5^a	74.5 ^b	1977.1 ^{ab}	312.5 ^b	543.8 ^b	752.4 ^b	877.5 ^b	279.2 ^b	888.8 ^{ab}	<0.001
Plant toxins	1994.1 ^b	61484 ^{ab}	12.2 ^b	1939.2 ^b	31074 ^{ab}	96428 ^a	27781 ^b	67092 ^{ab}	18225 ^b	6097.1 ^b	<0.001
Unspecific metabolites	799.8	1998.3	129	1641.4	4404.3	1648.5	1386	2261.3	2095.2	1498.3	0.125

^{ab} means with different letters identify groups that differ at $P < 0.05$, and $P < 0.10$ (when in brackets) from the highest value (in bold). Pairwise were compared according to the TUKEY-Kramer.

¹ Includes products of hay and lucerne cubes and flakes.

² Includes produces of grain-free mash or muesli.

³ Includes mineral feeds and treats.

Table 2: Concentration of mycotoxins, bacterial metabolites, lichen metabolites, phytoestrogens, plant metabolites, plant toxins and unspecific metabolites per product group (cont.)

Groups	Concentration (µg/kg)										P value
	Hay (n=5)	Processed plant products ¹ (n=24)	Grains (n=7)	Herbs (n=6)	Mash (n=5)	Mash GF ² (n=8)	Muesli (n=24)	Muesli GF ² (n=12)	Byproducts (n=11)	Others ³ (n=6)	
Sum of guidance value cate. mycotoxins	292.2 ^b	177.5 ^b	139.7 ^b	1003.7^a	179.5 ^b	230.3 ^b	143.8 ^b	140.4 ^b	82.3 ^b	495.6 ^b	<0.001
DON	223.7 ^b	143.2 ^b	52.8 ^b	993.6^a	87.2 ^b	224.1 ^b	73.1 ^b	120.5 ^b	47.3 ^b	487.5	<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
ZEA	19.8	15.8	1.8	0.7	7.5	6.1	25.6	7	33.6	2.5	0.652
Emerging mycotoxins	656.1	618.8	234.3	340.5	937	365	514.2	492	365.2	345.1	0.350
Culmorins	31.4	96.2	70.4	0	187.8	52.2	176.8	121	137.3	17.3	0.253
Enniatins	60	100.7	33.2	14.5	56.7	58.1	66.9	40.8	54.2	43.5	0.081
Siccanol	349.9	217.4	58.7	310.4	473.1	172.1	82.3	209.9	11.3	272.1	0.029

ab identify groups that significant differ ($P < 0.05$, and $P < 0.10$ when in brackets) from the highest value (in bold). Pairwise were compared according to the TUKEY-Kramer

¹ Includes products of hay and lucerne cubes and flakes.

² Includes produces of grain-free mash or muesli.

³ Includes mineral feeds and treats.

Table 3: Number of metabolites per group of origin (heat map)

Origin	Hay	Processed plant products															Grain					Byproducts					Herbs				
Alternaria spp	4 6 3 5 4	8 7 8 9 10 7 10 8 4 11 5 6 10 6 6 3 9 4 7 7 8 5 4 10	3 4 6 1 5 2 1	2 3 4 6 0 5 2 4 7 3 3	5 5 8 6 5 5																										
Aspergillus spp	3 10 5 3 2	11 10 7 11 12 7 10 8 5 12 5 6 8 8 7 4 9 10 10 7 4 8 6 11	0 0 0 3 0 0 0	3 1 0 1 1 9 2 4 5 2 2	3 4 7 8 8 9																										
Fusarium spp	12 21 12 22 18	24 20 18 23 28 15 25 18 17 34 17 9 25 14 14 10 16 12 20 14 15 17 11 21	24 14 27 15 6 18 3	9 5 26 10 15 17 13 17 14 18 16	11 10 14 11 15 8																										
Penicillium spp	4 3 3 3 1	8 7 7 16 12 6 11 7 5 13 6 4 8 10 6 4 5 2 8 6 2 9 5 8	2 2 1 5 1 2 0	1 1 2 1 1 4 2 5 5 14 1	6 6 15 4 6 4																										
Ergot alkaloids	0 0 0 0 0	8 0 0 1 11 8 2 0 0 12 0 0 1 5 6 4 4 0 8 8 0 10 0 8	0 0 0 0 0 0 0	0 0 12 0 0 3 0 3 0 0 0	0 0 0 0 0 0																										
Other fungi	6 12 9 10 7	14 14 14 17 16 14 21 18 13 22 13 9 16 8 9 9 8 9 17 12 9 16 10 18	6 2 4 2 2 2 2	8 0 1 3 3 11 7 2 13 7 4	7 4 12 11 7 7																										
Bacteria	0 0 0 0 0	2 1 3 0 3 2 3 2 2 2 2 0 2 1 0 0 0 0 1 1 0 1 0 0	0 0 0 0 0 0 0	0 0 0 0 2 1 0 0 0 0	1 2 2 0 0 1																										
Lichen	1 3 1 0 0	4 1 2 2 2 2 2 1 1 2 2 1 2 2 0 0 6 3 4 2 1 4 3 3	0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0 0	4 1 0 3 3 2																										
Phytoestrogens	8 7 3 5 6	8 8 8 8 8 8 9 8 5 9 7 7 8 9 9 9 8 5 8 7 5 8 8 8	2 5 6 3 3 0 1	1 3 4 9 3 9 1 2 9 1 0	0 1 1 1 7 4																										
Plant metabolites	1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1	1 1 1 0 1 1 1	0 1 1 1 0 1 1 0 1 1 1	1 1 0 1 1 1																										
Plant toxins	3 3 2 1 2	6 4 8 7 3 4 13 8 6 9 4 1 14 10 5 3 5 8 5 5 1 9 9 7	1 1 1 0 1 2 0	1 4 0 1 0 7 1 2 4 0 0	2 3 4 3 3 4																										
Unspecific origins	8 10 7 8 6	11 11 15 17 13 15 17 15 6 16 10 6 16 15 14 11 11 5 13 13 8 14 9 15	7 4 3 8 6 8 7	4 4 7 9 6 11 7 12 8 8 7	10 11 15 13 14 11																										
Alternaria spp	9 7 7 6 8	8 8 6 4 9 8 3 7 8 8 8 6 5 5 5 7 4 9 5 5 9 7 7 8	10 10 6 5 7 7 7 7	8 9 5 8 9 8 7 8 9 9 9 7	5 8 6 6 0 4																										
Aspergillus spp	12 4 7 10 9	7 7 3 8 6 6 6 9 3 10 7 4 4 2 3 5 5 7 3 5 7 8 5 7	12 10 4 6 7 6 6 6	10 12 7 11 10 6 6 7 8 6 11 6	7 9 10 7 0 3																										
Fusarium spp	27 25 16 19 21	21 24 29 32 28 35 24 17 30 13 22 20 18 26 21 16 15 27 23 16 19 22 27 26	22 16 9 13 13 16 18 9	13 25 16 18 13 14 25 14 24 26 21 10	12 9 14 16 0 9																										
Penicillium spp	11 6 6 11 13	7 14 3 8 11 9 9 6 9 5 11 4 7 10 8 8 8 13 3 6 7 11 11 11	10 9 6 7 9 9 9 5	6 15 7 6 4 6 14 8 11 12 9 9	5 12 10 9 0 2																										
Ergot alkaloids	0 7 0 3 3	4 3 4 12 9 12 4 6 8 8 8 6 9 9 2 10 8 8 6 5 10 4 8 6	7 9 7 4 8 3 7 3	10 1 6 4 6 2 8 1 6 7 9 4	9 0 0 0 0 5																										
Other fungi	21 10 10 7 14	16 8 4 14 14 12 10 17 5 16 13 11 9 4 4 20 8 11 5 6 15 15 12 10	17 17 12 13 13 19 15 15	13 17 12 16 16 10 13 9 21 13 20 13	6 10 7 7 0 2																										
Bacteria	2 0 0 0 0	2 0 0 0 2 1 0 2 0 2 1 2 0 0 0 0 0 0 0 0 0 1 0 0	2 2 2 2 2 0 0 0	2 1 2 4 2 0 0 0 0 0 0	2 0 1 1 0 0																										
Lichen	2 0 0 0 1	0 0 0 1 5 0 0 0 0 3 4 0 0 0 0 1 0 1 0 1 3 4 0 1	3 0 1 1 2 0 2 2	0 1 1 2 3 0 0 1 4 1 4 1	1 1 1 1 0 1																										
Phytoestrogens	4 8 8 9 8	9 6 8 9 9 9 10 9 9 8 9 9 8 8 8 8 9 9 10 8 8 8 9 8 10	8 8 8 8 8 9 8 8	8 8 8 8 8 9 9 8 9 9 8 8	9 7 8 8 0 1																										
Plant metabolites	1 1 1 1 1	1 0 1 1	1 1 1 1 1 1 0 1	1 1 1 1 1 1 1 1 0 1 1 1	1 1 1 1 0 0																										
Plant toxins	3 5 5 3 4	9 6 3 7 8 7 3 8 4 12 6 3 6 3 2 8 6 4 4 8 5 11 8 9	11 13 11 12 7 8 6 8	8 7 6 12 12 11 9 11 9 7 10 5	9 8 8 1 0 3																										
Unspecific origins	14 12 12 16 15	15 14 9 12 15 14 14 13 13 13 13 15 8 11 12 15 10 15 14 12 13 13 12 14	16 13 11 12 13 16 15 14	14 18 16 14 13 12 14 16 16 15 14 13	13 16 12 15 0 8																										
Origin	Mash	Muesli															Grain-free mash					Grain-free muesli					Mineral feed & others				

Table 4: Concentration of mycotoxins, bacterial metabolites, lichen metabolites, phytoestrogens, plant metabolites, plant toxins and unspecific metabolites in hay and processed plant products

Groups	Concentration ($\mu\text{g}/\text{kg}$)				SE	P value
	Hay (n=5)	Processed plant products				
		Gras ¹ (n=11)	Lucerne ² (n=8)	Mix ³ (n=5)		
Alternaria metabolites	10	2443	383.1	732.8	390.3	0.311
Aspergillus metabolites	735.8	147.4	193.6	263.7	117.5	0.412
Fusarium metabolites	1301.1	1452.5	744.2	1153.3	131.9	0.706
Penicillium metabolites	64.1	1194.7	304.9	321.4	215.0	0.439
Ergot alkaloids	0b	102.7^a	28.1 ^{ab}	29.5 ^{ab}	19.0	0.020
Other fungal metabolites	899.9^a	299 ^(b)	162 ^b	280.3 ^{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	34522 ^{ab}	49091^(a)	60939^a	10387.6	0.045
Plant metabolites	462.4 ^b	1183.5 ^b	3884.3^a	1724.6 ^b	638.3	<0.001
Plant toxins	1994.1 ^b	54477 ^{ab}	38545 ^(b)	113599^a	20127.9	0.017
Unspecific metabolites	799.8	2440.3	1286.1	2165.3	330.1	0.143

ab, Lsmeans sharing no common superscripts differ at $P < 0.05$ according to Tukey Kramer test, (b) indicates a tendency ($0.05 < P \leq 0.10$)

¹ *Includes hay cobs*

² *Includes lucerne cobs*

³ *Includes cobs based on a mix of gras and lucerne*

Table 5: The occurrence of mycotoxins, bacterial metabolites, lichen metabolites, phytoestrogens, plant metabolites, plant toxins and unspecific metabolites in hay vs. processed plant products

Groups	Average number of metabolites (n)				SE	P value
	Hay	Gras ¹	Lucerne ² Fehler! Textmarke nicht definiert.	MixFehler! Textmarke nicht definiert.		
Alternaria metabolites	4.4b	8.1a	5.4b	8.0a	0.8	0.002
Aspergillus metabolites	4.6b	9.1a	6.8ab	8.4(a)	0.9	0.015
Fusarium metabolites	17.0	20.0	14.8	19.8	1.1	0.216
Penicillium metabolites	2.8c	8.6a	5.0bc	8.0ab	1.2	0.003
Ergot alkaloids	0b	6.5a	1.3b	2.8ab	1.2	0.002
Other fungal metabolites	8.8b	14.8a	11.6ab	14.0ab	1.2	0.038
Bacterial metabolites	0b	1.1ab	0.75ab	2.0a	0.4	0.002
Lichen metabolites	1.0	2.3	2.0	2.2	0.3	0.404
Phytoestrogens	5.8c	8.3a	6.6b	8.2ab	0.5	0.001
Plant metabolites	1.0	0.9	1.0	1.0	0.0	0.674
Plant toxins	2.2b	6ab	5.3(b)	9.2a	1.2	0.006
Unspecific metabolites	7.8b	14.1a	8.6b	14.4a	1.5	<0.001

ab, Lsmeans sharing no common superscripts differ at $P < 0.05$ according to Tukey Kramer test, (b) indicates a tendency ($0.05 < P \leq 0.10$)

¹ *Includes hay cobs*

² *Includes lucerne cobs*

³ *Includes cobs based on a mix of gras and lucerne*

Table 6: Occurrence and concentration of pesticide and vet drug residues per product group

Groups	Concentration ($\mu\text{g}/\text{kg}$)							P value
	Processed plant products (n=22)	Grains (n=6)	Mash (n=5)	Mash GF (n=6)	Muesli (n=24)	Muesli GF (n=11)	Byproducts (n=9)	
Insecticide residues	34.3	40.9	97.4	19.1	121.4	38.2	29.3	0.318
Fungicide residues	44.8	35.9	193.9	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	291.3	182.6	246.4	109.5	111.7	0.039
Vet drug residues	111.6	0	303.1	0	81.2	384.7	0	0.678
Most frequently detected residues (>60% occurrence)								
Piperonylbutoxid	5.4 ^b	38.1 ^{ab}	80.9 ^{ab}	6.7 ^{ab}	102.3 ^a	13.1 ^{ab}	17.6 ^{ab}	0.047
DDAC	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
Number 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
Number of 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
Number of herbicide residues	0.4	0	0	0.2	0.1	0	0	0.056
Sum number of pesticides	2.9 ^b	2 ^b	10.8 ^a	8.2 ^a	6.7 ^a	6.8 ^a	4.2 ^b	<0.001
Number of vet drugs	0.3	0	1	0	0.6	0.6	0	0.392

ab, Lsmeans sharing no common superscripts differ at $P < 0.05$ according to Tukey Kramer test

¹ *Includes products of hay and lucerne cubes and flakes.*

² *Includes produces of grain-free mash or muesli.*

³ *Includes mineral feeds and treats.*

5. Discussion

In this study we analyzed the contamination with biotic and abiotic contaminants of commercial horse feed. The aim of this study was to determine if the contamination of horse feed with mycotoxins, bacterial metabolites, lichen metabolites, phytoestrogens, plant metabolites, plant toxins and unspecific metabolites is correlated with specific types of horse feed or ingredients of the product. Furthermore, we estimated whether the contamination load of the products might pose a risk to the horse's health.

5.1. Material and method

This study included 108 horse feed products available on the market in five different countries. The products were randomly chosen and sent to the laboratory for analyses, where they were analyzed for over 250 contaminants. To our best knowledge, there is no other study that investigated such a broad spectrum of contaminants including biotic and abiotic contaminants.

5.2. Feed regulations

Despite general feed regulations for all animals, such as the ones mentioned in EU regulation (EC) No. 767/2009, contaminants in equine feed stuff remain mostly unregulated. Especially when compared to livestock feed, which underlie a more stringent legal regulation due to their direct connection to human food sources. For equine feed, specific thresholds are limited, with only fumonisin B1+B2 regulated under EU guidelines. This mycotoxin when ingested in high concentrations by horses leads to Equine Leukoencephalomalacia (16). For instance, other *Fusarium* toxins, such as deoxynivalenol (DON) and zearalenone, especially as co-contaminants may lead to reduced feed intake and reproductive health issues (19,20). However, DON and zearalenone lack established safety thresholds for horses, despite documented impacts on livestock, highlighting an area in need of regulatory development. This regulatory gap permits the presence of various toxins, including unregulated mycotoxins and plant-based contaminants, which may cumulatively pose health risks to horses.

5.3. Biotic contaminants

The study's detection of multiple mycotoxins, particularly metabolites from *Fusarium*, *Alternaria*, and *Penicillium* fungi, across nearly all tested feed samples aligns with concerns documented in prior research (3). These findings suggest a consistent susceptibility of equine feeds to fungal contamination. *Fusarium* metabolites are the most frequent contaminants across all types of feedstuffs we analyzed. Our findings of widespread *Fusarium* mycotoxins in horse feed echo Ensley and Mostrom's observation that mycotoxins like fumonisins and deoxynivalenol (DON) are prevalent in equine diets, particularly in grains such as corn and

oats (40). This aligns with findings that showed mycotoxins were prevalent in over 80% of hay samples in the UK, with Durham notably identifying fumonisin B1, deoxynivalenol, and zearalenone exclusively in premises experiencing liver disease outbreaks (41). This suggests a potential link between these mycotoxins and equine health issues, reinforcing the need for caution in feed management. Newman notes that factors such as environmental conditions, feed storage, and the presence of multiple mycotoxins can exacerbate the risk of mycotoxicosis, as well as that chronic low-level exposure to mycotoxins can lead to subclinical issues such as immune suppression and decreased performance, even in the absence of overt toxicity symptoms (42). This is particularly relevant for performance and breeding horses, where subtle health impacts can significantly affect athletic and reproductive outcomes. Our study's detection of mycotoxins across various feed types suggests that horses are consistently exposed to these contaminants, warranting further investigation into their long-term health implications.

Guidance value mycotoxins were found in significantly higher concentrations in herbs than in other feed products. Of these, only fumonisin B1+B2 is currently regulated by the EU Recommendation 2016/1319, with a guidance level set for horses. Specifically, the maximum allowed level in horse feed is up to 5 mg/kg of fumonisin B1+B2 at a 12 % moisture content. The documented link between fumonisins and equine leukoencephalomalacia underscores the potential risks posed by contaminated feed. The highest concentration we detected was 0.052 mg/kg in a cereal product, which is well below the guidance value. Grains are more susceptible to fungal growth because they are rich in carbohydrates, proteins, and other nutrients that provide an ideal substrate for fungal growth. This nutrient density supports the proliferation of fungi, particularly those that produce mycotoxins. During growth, harvest, and storage, grains can retain moisture, creating a conducive environment for fungal growth. High humidity and moisture levels are critical factors that promote fungal colonization and mycotoxin production. The structure of grains, with their protective husks and kernels, can trap moisture and create microenvironments that favor fungal growth. Any damage to the grain's outer layers during harvesting or handling can further increase susceptibility by providing entry points for fungal spores.

DON, on the other hand, does not have a maximum allowable concentration for horses. However, the highest concentration permitted in swine feed is 0.9 mg/kg, and for ruminants, it is 2 mg/kg. In our study, the highest DON concentration found was 2.2 mg/kg in a processed plant product, which exceeds the guidance level set for ruminants. Looking at all feeding products herbs showed significantly higher concentrations of all guidance value mycotoxins. The reason for that might be that moisture can be retained if the herbs have broad leaves and dense growth patterns. Moreover, herbs are often air dried and do not undergo more processing treatment, which often include heat treatments. Although research suggests that

DON has a more significant impact on the health of pigs and ruminants than on horses, it would be prudent to consider adapting these established guidance levels for horse feed products, especially since the effects of co-contaminations have not yet been fully evaluated (18). The highest concentrations of Zearalenone we found were in Byproducts and Muesli but with no significant difference to other feeds. But as Vance et al. demonstrate that chronic exposure to Zearalenone, even at low doses, can influence reproductive parameters in mares, such as progesterone levels and pregnancy rates (43). Our study's detection of mycotoxins like Zearalenone in horse feed suggests that certain feed ingredients may pose similar risks. This underscores the importance of monitoring and managing mycotoxin levels in equine diets to prevent potential reproductive issues. The study's findings on the variability of reproductive responses to Zearalenone exposure highlight the need for further research into the long-term effects of mycotoxins in equine diets. This aligns with our call for comprehensive studies on the cumulative impacts of multiple contaminants, including mycotoxins. Developing guidelines for acceptable levels of mycotoxins in equine feed can enhance safety and protect horse health.

Our study's detection of multiple mycotoxins, particularly deoxynivalenol (DON) and zearalenone, is consistent with Schumann et al., who demonstrated that DON and Zearalenone, along with their metabolites, could significantly reduce the viability and proliferation of equine immune cells *in vitro* (22). This highlights the potential for these mycotoxins to compromise immune function, even at low exposure levels, which is particularly concerning given their prevalence in horse feed. Notably, Schumann et al. also reported that DON and its acetylated form exhibit lower IC₅₀ values compared to zearalenone and its metabolites, indicating a higher cytotoxicity of DON to equine immune cells (22).

These results are further supported by the work of Songsermsakul et al., who investigated the metabolism of zearalenone in horses fed *Fusarium*-contaminated oats (21). They found that horses primarily metabolize zearalenone into β -zearalenol (β -ZOL), in contrast to other species, such as swine, where α -zearalenol (α -ZOL) is the dominant metabolite. This metabolic difference may explain why horses exhibit a unique sensitivity to zearalenone compared to pigs, which are more susceptible to its estrogenic effects. Additionally, Songsermsakul et al. highlighted the extensive glucuronidation of zearalenone and its metabolites in horses, with nearly complete conjugation observed in plasma and urine (21). While this suggests an efficient detoxification mechanism that may mitigate adverse effects, the persistent detection of zearalenone and its metabolites in biological matrices signals a continuous exposure risk. These findings highlight the importance of monitoring and managing mycotoxin levels in equine diets to protect horse health.

The immunosuppressive effects observed *In vitro* suggest that these mycotoxins could reduce resilience to infections and stress, impacting overall performance (23). This emphasizes the need for comprehensive monitoring and management practices to mitigate these risks, particularly in high-performance equine environments. The study by Dänicke et al. also highlighted the role of mycotoxins in exacerbating intestinal inflammation and compromising the mucosal barrier, which could facilitate the transfer of toxins into systemic circulation (23). This mechanism may explain the higher incidence of DON in colic-affected horses, as intestinal lesions could enhance toxin absorption. The study's association between DON exposure and increased activities of liver-related enzymes, such as aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT), indicates possible liver involvement in mycotoxin metabolism and its role in systemic inflammation (23). The co-occurrence of fusariotoxins with other contaminants, such as pesticides, raises concerns about potential synergistic effects due to the complex interactions between fusariotoxins and other compounds, which can exacerbate their toxic effects (19). This is particularly relevant given our findings of multiple contaminants in equine feed, suggesting a need for studies exploring these interactions and their cumulative impact on equine health.

In this study we found significantly higher concentrations of ergot alkaloids in grain-free muesli than in the other feed stuff. This is interesting since *Claviceps* spp., which produce ergot alkaloids are commonly associated with grains like rye and triticale, but it can also be found on rye grass or other grasses which might be the source in these products. Our study found a significantly higher concentration of *Aspergillus* metabolites, including Aflatoxins, and *Penicillium* metabolites in mash than in other horse feed. The reason for that might be that mash ingredients, such as molasses, can retain higher moisture compared to dry feeds, making them more susceptible to *Aspergillus* and *Penicillium* growth. Moreover, mash contains grains which are also more susceptible to fungal growth, like discussed earlier. Furthermore, *Penicillium* is known to be found on feed that has begun to deteriorate. Again, due to the high moisture and nutrient content in mash, this product might degrade more quickly. The consequences of Aflatoxicosis are known. Like the findings of Braga et al., who investigated the effects of mycotoxins on horses during short-term exercises by demonstrating that the presence of aflatoxin B1 (AFB1) in horse diets significantly influenced alkaline phosphatase activity, suggesting a hepatotoxic impact (12). This aligns with our findings, where mycotoxins like fumonisin B1 were prevalent, raising concerns about their potential cumulative effects on liver health. They observed that while AFB1 did not significantly alter most physiological parameters during exercise, it did increase alkaline phosphatase activity at higher concentrations, indicating possible liver stress (12). This suggests that even subclinical levels of mycotoxins could impact equine health, particularly under conditions of physical exertion. Our detection of multiple mycotoxins, including DON and zearalenone, known for their

synergistic effects, underscores the need for further research into their combined impacts on equine physiology and performance.

Furthermore, in this study a higher concentration of lichen metabolites was found in herbs, but some herb products contain plants which belong to the lichen genus.

5.4. Abiotic contaminants

Additionally, the detection of abiotic contaminants, including pesticide residues like piperonyl butoxide and tebuconazole, shows the environmental factors influencing feed safety. Notably, these pesticides may enter the feed supply through agricultural practices on fields where feed ingredients are cultivated, or they may occur due to residual contamination during feed production. Significantly higher numbers of pesticides, especially insecticides and fungicides were found in mash, grain-free mash, muesli and grain-free muesli. This again might be because of the higher complexity of ingredients in compound feeds and multiple possible 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. Plumlee emphasizes that inappropriate handling or accidental ingestion of pesticide-contaminated feed can lead to toxicosis, even at low exposure levels (44). Misuse and accidental exposure are primary causes of toxicosis, suggesting that improved handling and storage practices could mitigate these risks (45,46). Given their recognized impacts on non-target species, including cytotoxicity in human alveolar cells and developmental risks in mammals, it might hint to contamination risks for horses (34–37). But the specific effects of these pesticides on horses are yet to be investigated. The most frequently detected pesticide residues were piperonyl butoxide, DDAC, and tebuconazole, which were present in about 65 % of the 83 samples analyzed. Moreover, pesticide residues were found in higher concentrations in supplementary feeds than in processed plant products and byproducts, with fungicide residues being the most common. As observed in our study, pesticide residues were more prevalent in processed feeds. This is consistent with findings, which highlight that the toxicity of pesticides can vary but often results in adverse health effects, including central nervous system impacts, even at sublethal doses (44). Given that pesticide toxicosis, by anticholinesterase insecticides, metaldehyde, zinc phosphide can lead to significant health issues in horses, including respiratory distress and neurological symptoms (44), it underscores the urgent need for stricter regulatory thresholds for pesticide residues in equine feed. Plumlee notes that while many herbicides have low toxicity in mammals, they can indirectly cause toxicosis by altering the toxicity of poisonous plants (44). This insight is particularly relevant given our study's detection of plant-based contaminants. The potential for herbicides to increase the palatability or toxin concentration of certain plants suggests a need for

comprehensive safety evaluations of feed ingredients, especially those sourced from treated pastures.

Veterinary drug residues were rarely detected, with monensin and dinitrocarbanilide being the most common, found in approximately 10 % of the samples. Monensin is an anticoccidial drug used for ruminants and poultry, but horses are much more sensitive to it, with a median lethal dose of just 2–3 mg/kg of body weight (38). Horses which ingested monensin show symptoms like anorexia, ataxia and sweating (38). The 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. Since the moisture level of this feed was not established, it is unclear whether this concentration exceeds the legal limit. Horses can be fed 200–400 g of this product per 100 kg of body weight. Two other products from Hungary contained 0.2 mg/kg of monensin: one was a cereal product, recommended to be fed at 400–1200 g per 100 kg of body weight, and the other was a mash, with feeding instructions of 50–200 g per 100 kg of body weight. As shown in Box 2, this level would not be lethal for horses.

Plant toxins were found in a significantly higher concentration in grain-free mash than in other horse feeds. Especially high concentrations were Colchicine, which is a toxin found in the toxic plant meadow saffron (*Colchicum autumnale*). A dose of 1 mg of colchicine per kg of body weight can be lethal for a horse (26,27). Feeding hay contaminated with *C. autumnale* is known to pose a risk of intoxication. 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 from Hungary, which has a feeding recommendation of 50–200 g per 100 kg of body weight. The second-highest concentration detected was 8.4 mg/kg in a processed plant product from Hungary, intended to be fed at a rate of 1000–1500 g per 100 kg of body weight. Another notably high concentration was 4.7 mg/kg in a grain-free cereal product from Hungary, which can be fed at 200–400 g per 100 kg of body weight. All other samples contained colchicine at concentrations below 0.05 mg/kg of feed. As shown in Box 1, these concentrations should not pose a direct risk to a horse's health, as even the two products with the highest concentrations are significantly below the lethal dose and also below one-quarter of the dose known to cause diarrhea. However, in regions where meadow saffron is widespread, it may be difficult to avoid feeding hay contaminated with this toxic plant, raising concerns about accumulation and long-term effects. Although meadow saffron is a well-known toxic plant, with established lethal doses and symptoms, colchicine was still detected in one-fifth of the tested feed products.

5.5. Comparison of feed products

Out of the 108 horse feed products analyzed, only one was found to be contaminated. This was a single feed product composed entirely of methylsulfonylmethane, a white crystalline substance. Since this product is the only one not derived from any type of plant, grain, or tuber, it may be less susceptible to contamination; however, this study could not effectively determine the cause.

Although we were not yet able to identify specific ingredients that trigger the occurrence of contaminants, we found that single-ingredient feeds, like hay, grains, and byproducts, generally have a lower concentration and number of mycotoxins and pesticide residues. Compound feeds, which blend diverse ingredients to meet the elevated energy and nutrient requirements of performance or breeding horses, appear to increase contamination risk. Additionally, compound feeds tend to have more complex profiles of fungal contaminants compared to single feedstuffs. This shows the need for further considerations regarding feed composition and production processes. The complexity of compound 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. Moreover, single ingredient products often originate from one source again making controls easier and the process of production less complicated and reducing the risk of contamination. Also, the moisture content of the different ingredients of compound feed might differ and therefore positively enhance fungal growth.

When comparing roughage, we observed that processed plant products generally exhibit higher concentrations of plant metabolites, phytoestrogens, and plant toxins than hay. Phytoestrogens are known to lead to reproductive issues in horses, but the cause of high concentration is not alone the plant composition of a pasture (2,32). Wyse et al. highlight that phytoestrogens are prevalent in legume species, which are common in equine diets (47). These compounds can have both beneficial and adverse effects, depending on concentration and exposure duration (47). Our observation that processed plant products exhibit higher concentrations of phytoestrogens than hay is consistent with the review's findings. This suggests a potential risk for reproductive issues, such as uterine edema and anovulation in mares, as noted in the review. Therefore, selecting feed ingredients with lower phytoestrogen content may be crucial for managing reproductive health in horses. Moreover, grass based processed plant products exhibit a greater number of metabolites throughout nearly all contaminant groups. This highlights the importance of investigating effects of co-contaminations. However, hay contains a greater variety of metabolites from fungi other than *Alternaria*, *Aspergillus*, *Fusarium*, *Penicillium*, and ergot-producing fungi. Ergot alkaloids, which weaken the reproductive ability in horses and might also cause other symptoms given

the knowledge of their high toxicity in other mammals, were found in higher concentrations in grass-based forages compared to hay and Lucerne-based products (13,14). Since there are no established concentration thresholds known to trigger effects on a horse's body, we cannot definitively assess whether the concentrations found in this study are hazardous.

5.6. Outlook

To sum up, over 90 % of the samples showed various co-contaminations with biotic and abiotic contaminants. The most frequent contaminant groups were mycotoxins and pesticide residues. Even though, we were not yet able to determine specific ingredients which cause a higher contaminant load, we discovered that single feed stuff generally has lower concentrations and number of mycotoxins and pesticide residues than compound feed and processed plant products have higher concentrations of plant metabolites, phytoestrogens and plant toxins than hay. Although no product in this study seemed to have a critical concentration of the contaminants known to have negative effects on horse's health, this study revealed that every product showed co-contaminations with different contaminant groups, and we cannot yet assess the risk of co-contamination. To the best of our knowledge, no study has yet investigated the impact of contamination with multiple toxins on horses. The co-occurrence of multiple mycotoxins in feed samples raises concerns about cumulative toxicity. Ensley and Mostrom emphasize 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 (40). Surprisingly, for most contaminants, there are no studies that have determined the potential consequences for horses. Even for the few toxins where the effects are known, the concentrations that lead to health problems have not been thoroughly investigated. That's why, this study highlights the necessity for additional research into the effects of co-contaminants on equine health, as the cumulative impacts of low-dose mycotoxins, plant toxins, and pesticides remain largely uncharted. Thus, further studies examining these synergistic effects could significantly inform safe feeding practices and regulatory standards.

Box 1: Sample calculation of colchicine for a 600 kg horse

Colchicine limit value in the body: $1 \frac{mg}{kg} \rightarrow 600mg$

Sample 31: Mash

Feeding instruction: $\frac{200 g}{100 kg} \rightarrow \frac{0.2 kg}{100 kg}$

Colchicine in the product: $29.6 \frac{mg}{kg}$

Amount of colchicine in standard feed quantity

$$600 kg \cdot \frac{0.2 kg}{100 kg} \cdot 29.6 \frac{mg}{kg} = 35.52 mg < 600mg$$

Sample 32: Processed plant product

Feeding instruction: $\frac{1500 g}{100 kg}$

Colchicine in product: $8.4 \frac{mg}{kg}$

Amount of colchicine in standard feed quantity

$$600 kg \cdot \frac{1.5 kg}{100 kg} \cdot 8.4 \frac{mg}{kg} = 75.6 mg < 600mg$$

Box 2: Sample calculation of monensin for a 600 kg horse

Monensin limit value in the body: 2 to 3 $\frac{mg}{kg} \rightarrow 1200$ to 1800 mg

Sample 29: Grainfree muesli

Feeding instruction: $\frac{400 g}{100 kg}$

Monensin in the product: $1.9 \frac{mg}{kg}$

Amount of monensin in standard feed quantity

$$600 kg \cdot \frac{0.4 kg}{100 kg} \cdot 1.9 \frac{mg}{kg} = 4.56 mg$$

Sample 28: muesli

Feeding instruction: $\frac{1200 g}{100 kg}$

Monensin in product: $0.2 \frac{mg}{kg}$

Amount of monensin in standard feed quantity

$$600 kg \cdot \frac{1.2 kg}{100 kg} \cdot 0.2 \frac{mg}{kg} = 1.44 mg$$

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Appendix

Table A: Overview of feed samples

Single/compound	Type of product	Name	Provider	Country
Single feed	Hay	Heuprobe Schilfwiese	Region Künzelsau	DE
Single feed	Hay	Heuprobe Schellenbergwiese	Region Künzelsau	DE
Single feed	Hay	Heuprobe Pferdekoppel	Region Künzelsau	DE
Single feed	Hay	Heuprobe Brunnenwiese	Region Künzelsau	DE
Single feed	Hay	Heuprobe Kesselfeld Pferdekoppel	Region Künzelsau	DE
Single feed	Processed plant products	Luzerne	Makana Produktion und Vertrieb GmbH	DE
Single feed	Processed plant products	Luzerner-Brok Havens Graanhandel	Havens Graanhandel NV	DE
Single feed	Processed plant products	Luzerne Pellets		FR
Single feed	Processed plant products	Höveler Pur.Luzerne Pellets	Höveler Pferdefutter EQUOVIS GmbH	DE
Single feed	Processed plant products	Agrobs Pre Alpin Wiesencobs	Agrobs GmbH	CH
Single feed	Processed plant products	Fixkraft Luzerne Cobs	Fixkraft Futtermittel GmbH	AT
Single feed	Processed plant products	HippoSport Luzernecobs Bio	HippoSport GmbH	DE
Single feed	Processed plant products	Luzatop- Luzerne	Desialis	CH
Single feed	Processed plant products	Luzerne Quebag	QUEBAG AG	DE
Single feed	Processed plant products	Nordic Grass - Heucobs	Halmeko LLC	HU
Single feed	Processed plant products	Tocks- Tocks-Cobs	Futtermühle Tock GmbH	CH
Single feed	Processed plant products	Heucobs Quebag	QUEBAG AG	DE
Single feed	Processed plant products	Heucobs Deukavallo	Deutsche Tiernahrung Cremer GmbH & Co. KG / deuka	DE
Single feed	Processed plant products	Marstall Heucobs	marstall GmbH	DE

Table A: Overview of feed samples (cont.)

Single/compound	Type of product	Name	Provider	Country
Single feed	Processed plant products	Derby Bergwiesencops	EQUOVIS GmbH /Derby	DE
Compound feed	Processed plant products	Fixkraft Elite Heuflakes	Fixkraft Futtermittel GmbH	HU
Compound feed	Processed plant products	Agrobs Kraftpaket	Agrobs GmbH	DE
Compound feed	Processed plant products	Agrobs Kraftpaket	Agrobs GmbH	AT
Compound feed	Processed plant products	Agrobs Pre Alpin Senior	Agrobs GmbH	CH
Compound feed	Processed plant products	Agrobs Pre Alpin Senior	Agrobs GmbH	CH
Compound feed	Processed plant products	Helianthus Heucobs	Vestmann Distributors Limited / Helianthus Horse Feed	HU
Compound feed	Processed plant products	BioSuisse- Bio Graswürfel	Bio Suisse	CH
Compound feed	Processed plant products	Fixkraft Elite Heuflakes	Fixkraft Futtermittel GmbH	CH
Compound feed	Processed plant products	Fixkraft Elite Heuflakes	Fixkraft Futtermittel GmbH	AT
Single feed	Grains	Pavo Hafer	PAVO Pferdenahrung GmbH	DE
Single feed	Grains	Maisflocken trocken		AT
Single feed	Grains	Hafer Bio		DE
Single feed	Grains	Hypona- Hafer	UFA AG HYPONA	CH
Single feed	Grains	Hafer		AT
Single feed	Grains	Marstall Gerste	marstall GmbH	DE
Single feed	Grains	Raiffeisen eigen gequetschte Gerste	Raiffeisenbank Ems Vechte eG	DE
Single feed	Herbs	Weißdorn	Masterhorse GmbH	DE
Single feed	Herbs	Lexa Weiderinde	Xaver Scheule GmbH / LEXA	DE
Compound feed	Herbs	Lüneburger Kräuter	PerNaturam GmbH	DE
Compound feed	Herbs	Lexa Kräuter für Haut und Fell	Xaver Scheule GmbH / LEXA	DE
Compound feed	Herbs	Bitterkräuter Fine		DE
Compound feed	Herbs	Mordskerl Mineralisierung Pulver	Dr. Weyrauch Kräuterspezialitäten	DE

Table 1: Overview of feed samples (cont.)

Single/compound	Type of product	Name	Provider	Country
Compound feed	Mash	St. Hippolyt Irish Mash	St. Hippolyt Mühle Ebert GmbH	DE
Compound feed	Mash	Landmühle British Mash	Krämer Pferdesport GmbH&Co.KG	CH
Compound feed	Mash	Helianthus Fibra Mash	Vestmann Distributors Limited / Helianthus Horse Feed	HU
Compound feed	Mash	Sighorse Schmankerl Mash	Siglmühle GmbH	AT
Compound feed	Mash	Marstall Mash	marstall GmbH	CH
Compound feed	Grain-free Mash	Agrobs Alpengrün Mash getreidefrei	Agrobs GmbH	DE
Compound feed	Grain-free Mash	Agrobs Alpengrün Mash getreidefrei	Agrobs GmbH	HU
Compound feed	Grain-free Mash	Agrobs Alpengrün Mash getreidefrei	Agrobs GmbH	CH
Compound feed	Grain-free Mash	Agrobs Alpengrün Mash getreidefrei	Agrobs GmbH	AT
Compound feed	Grain-free Mash	Agrobs Alpengrün Mash getreidefrei	Agrobs GmbH	DE
Compound feed	Grain-free Mash	HBDs Equidietic (Mash)	Heiner Beifuss Dielheim e.K.	DE
Compound feed	Grain-free Mash	St. Hippolyt Glyx-Mash (getreidefrei)	St. Hippolyt Mühle Ebert GmbH	DE
Compound feed	Grain-free Mash	Marstall Bergwiesen Mash	marstall GmbH	CH
Compound feed	Muesli	Fixkraft Elite Senior Gut Aiderbichl Müsli	Fixkraft Futtermittel GmbH	HU
Compound feed	Muesli	Marstall Kräuterwiese	marstall GmbH	CH
Compound feed	Muesli	Senior Müsli Cavalor	Nutriquine NV / Versele-Laga NV / Cavalor NA // Cavalor	DE
Compound feed	Muesli	Fixkraft Elite Senior Gut Aiderbichl Müsli	Frixkraft Futtermittel GmbH	AT
Compound feed	Muesli	St. Hippolyt Struktur E Getreidefrei	St. Hippolyt Mühle Ebert GmbH	CH
Compound feed	Muesli	St. Hippolyt Struktur E Energetikum	St. Hippolyt Mühle Ebert GmbH	DE
Compound feed	Muesli	Marstall Zuchtmüsli für Zuchtstuten und Deckhengste	marstall GmbH	DE
Compound feed	Muesli	Marstall Stehfutter	marstall GmbH	CH

Table 1: Overview of feed samples (cont.)

Single/compound	Type of product	Name	Provider	Country
Compound feed	Muesli	Helianthus Senior	Vestmann Distributors Limited / Helianthus Horse Feed	HU
Compound feed	Muesli	Marstall Isländer Robust Müsli	Equsana	AT
Compound feed	Muesli	Agrobs Horse Alpin Senior	Agrobs GmbH	DE
Compound feed	Muesli	Graf's Knuspermüsli	Johann Graf GmbH	DE
Compound feed	Muesli	Dodson&Horell Build Up Conditioning	Dodson & Horrell Ltd	DE
Compound feed	Muesli	St. Hippolyt Beste Jahre Müsli	St. Hippolyt Mühle Ebert GmbH	AT
Compound feed	Muesli	Lexa Vitalmix Bio	Xaver Scheule GmbH / LEXA	CH
Compound feed	Muesli	Marstall Senior Plus	marstall GmbH	CH
Compound feed	Muesli	Marstall Complete	marstall GmbH	CH
Compound feed	Muesli	Derby Countrymix	EQUOVIS GmbH /Derby	DE
Compound feed	Muesli	Sighorse Mutterliebe	Siglmühle GmbH	AT
Compound feed	Muesli	Sighorse Nimms Leicht	Siglmühle GmbH	AT
Compound feed	Muesli	Lavisano Green Probiotic	Lavisano Vertriebs GmbH	CH
Compound feed	Muesli	Lavisano Green Probiotic	Lavisano Vertriebs GmbH	DE
Compound feed	Muesli	Eggersmann Kombi Pellets	Heinrich Eggersmann Futtermittelwerke GmbH	DE
Compound feed	Muesli	Schafmüsli 17	Fixkraft Futtermittel GmbH	HU
Compound feed	Grain-free Muesli	Agrobs Alpengrün Müsli getreidefrei	Agrobs GmbH	DE
Compound feed	Grain-free Muesli	Agrobs Alpengrün Müsli getreidefrei	Agrobs GmbH	CH
Compound feed	Grain-free Muesli	St. Hippolyt RiceLein	St. Hippolyt Mühle Ebert GmbH	DE
Compound feed	Grain-free Muesli	Helianthus Sensicare-active	Vestmann Distributors Limited / Helianthus Horse Feed	HU
Compound feed	Grain-free Muesli	St. Hippolyt Struktur E Getreidefrei	St. Hippolyt Mühle Ebert GmbH	CH

Table 1: Overview of feed samples (cont.)

Single/compound	Type of product	Name	Provider	Country
Compound feed	Grain-free Muesli	St. Hippolyt Struktur E Getreidefrei	St. Hippolyt Mühle Ebert GmbH	DE
Compound feed	Grain-free Muesli	Höveler Puritan Pur Senior	Höveler Pferdefutter EQUOVIS GmbH	DE
Compound feed	Grain-free Muesli	Agrobs Alpengrün Müsli getreidefrei	Agrobs GmbH	DE
Compound feed	Grain-free Muesli	Marstall Senior Aktiv	marstall GmbH	AT
Compound feed	Grain-free Muesli	Marstall Faser-Light	marstall GmbH	AT
Compound feed	Grain-free Muesli	Lexa Strukturmix getreidefrei	Xaver Scheule GmbH / LEXA	CH
Compound feed	Grain-free Muesli	St. Hippolyt Glyx-Wiese/Seniorfaser	St. Hippolyt Mühle Ebert GmbH	DE
Single feed	Byproducts	Weizenkleie		AT
Single feed	Byproducts	Landi Agro- Weizenkleie	LANDI Schweiz AG	CH
Single feed	Byproducts	Zuckerrüben Pellets		AT
Single feed	Byproducts	Pavo Speedibeet	PAVO Pferdenahrung GmbH	CH
Single feed	Byproducts	Pavo Speedibeet	PAVO Pferdenahrung GmbH	AT
Single feed	Byproducts	Raiffeisen eigen Zuckerrübenschnitzel	Raiffeisenbank Ems Vechte eG	DE
Single feed	Byproducts	Rübenschnitzel		DE
Single feed	Byproducts	Pavo Speedibeet	PAVO Pferdenahrung GmbH	DE
Single feed	Byproducts	Optimera Sojaschrot	Optimera AG	CH
Single feed	Byproducts	Leingold zum Füttern	Schäfer	DE
Compound feed	Byproducts	Optibeets	Optimera AG	CH
Single feed	Mineral feed ¹	Lexa MSM	Xaver Scheule GmbH / LEXA	DE
Compound feed	Mineral feed ¹	HBDs Horse Mineralfutter	Heiner Beifuss Dielheim e.K.	DE
Compound feed	Mineral feed ¹	Beinhart Mineralisierung Pulver	Dr. Weyrauch Kräuterspezialitäten	DE
Compound feed	Mineral feed ¹	Equi Power Mineral	Vetripharm	DE
Compound feed	Mineral feed ¹	Farriers Formula Double Concentrat für die Hufe	Life Data LABS, Inc.	DE
Compound feed	Treats ¹	Leckerlie Energiebarren Kanne	Kanne Brottrunk GmbH & Co. Betriebsgesellschaft KG	DE

¹ Product group is categorized as "Others" in the analysis.