agriculture

Communication

Mycotoxin Contamination of Selected Organic Enrichment Materials Used in Pig Farming

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Received: 2 November 2020; Accepted: 19 November 2020; Published: 21 November 2020

Abstract: Abnormal behavior, such as tail biting, is a fundamental problem in pig husbandry worldwide, and the application of enrichment materials, particularly organic materials, is one of the most promising preventive and curative measures. However, the potential health risks posed by these materials, such as being an additional source of mycotoxins, have not been sufficiently studied to date. Therefore, 21 different organic enrichment materials were tested for mycotoxin contamination with a liquid chromatography tandem mass spectrometry multi-mycotoxin method. Concerning the legally regulated mycotoxins in the EU, aflatoxin B_1 and ochratoxin A were not detected in any of the tested materials. Fumonisin B_2 was detected in straw meal made of wheat, rye, and triticale, but the level (0.014 mg/kg) was very low. The level of deoxynivalenol in maize pellets (5.01 mg/kg) and maize silage (2.12 mg/kg) exceeded the guidance value for pig feed. Zearalenone was present at high levels in maize pellets (1.21 mg/kg), hay (0.30 mg/kg), and maize silage (0.25 mg/kg). Maize products showed high levels of mycotoxins presenting a health risk for pigs and cannot be recommended as enrichment material.

Keywords: deoxynivalenol; fumonisin; fungi; maize; ochratoxin; zearalenone

1. Introduction

In intensive pig production, tail biting is a frequent problem worldwide subsequently impairing the animal's welfare, health, and resilience while also leading to economic losses for the farmer [1]. A major risk factor for the outbreak of tail biting is a lack or insufficiency of manipulable enrichment material [2]. The administration of enrichment material is both preventive and curative for abnormal behavior such as tail biting [3]. The European Union Council Directive 2008/120/EC [4] requires farmers to provide their pigs with permanent access to a sufficient quantity of enrichment material. Commission Recommendation 2016/336/EU [5] specifies that the material should be edible, chewable, investigable, and manipulable; thus, only organic materials can be recommended as optimal enrichment for pigs. A review comparing different enrichment materials in their effectiveness and acceptance by pigs summarized the advantages of organic materials [6]. However, a further requirement that has to be fulfilled by the material is the safety concerning potential animal health effects [4,5]. Some materials such as straw can be contaminated with mycotoxins [7].

Mycotoxins are produced by fungi as secondary metabolites either on the field (e.g., *Fusarium* species) or during storage (e.g., *Aspergillus* and *Penicillium* species) [8]. The growth of molds and the amount of mycotoxin production depend on numerous physical, chemical, and biological factors with moisture and temperature being the most important [9]. Mycotoxins are commonly detected in animal feed and feed raw materials around the globe [10]. In farm animals, mycotoxins can have different toxic effects depending on the quantity and variety of mycotoxins, the age of the animals, and other predisposing factors such as stress and immune status [9,11]. Pigs are particularly sensitive to aflatoxin B_1 [12], fumonisin B_1 [11], deoxynivalenol (DON), zearalenone (ZEN), and ochratoxin A [8]. Well-known clinical signs of mycotoxicoses in pigs are gastrointestinal disorders or feed refusal and growth retardation induced by DON, reproductive disorders due to ZEN, mycotoxic nephropathy due to ochratoxin A, and the porcine pulmonary edema caused by fumonisin B_1 [8]. Hence, the European Union has set maximum permissible values and guidance values for mycotoxins [13,14]. However, in most cases, the effects are subclinical, e.g., impaired growth performance, reproduction, and immunity due to low levels of contamination [9].

To consider these potential subclinical effects, the European Food Safety Authority (EFSA) implemented no-observed-(adverse)-effect levels (NO(A)ELs) and lowest-observed-(adverse)-effect levels (LO(A)ELs) for DON [15], fumonisins [16], ochratoxin A [17], and ZEN [18]. NO(A)ELs are defined as the highest concentrations of mycotoxin in feed that have no (adverse) health effects. LO(A)ELs are the lowest concentrations of mycotoxin in feed that have an (adverse) health effect in a particular species, respectively. The maximum permissible values, guidance values, NO(A)ELs, and LO(A)ELs are summarized in Table 1.

Table 1. Maximum permissible values (mg mycotoxin/kg feed, based on a dry matter content of 88%), guidance values (mg mycotoxin/kg feed, based on a dry matter content of 88%), no-observed-(adverse)-effect levels (NO(A)ELs) (mg mycotoxin/kg feed), and lowest-observed-(adverse)-effect levels (LO(A)ELs) (mg mycotoxin/kg feed) for aflatoxin B_1, deoxynivalenol, zearalenone, ochratoxin A, and fumonisin B_1 and B_2.

Mycotoxin	Maximum Permissible Value [1]	Guidance Value [2]	NO(A)EL	LO(A)EL
aflatoxin B_1	0.02 (complete/complementary feedingstuffs for pigs except young animals) 0.01 (complete feedingstuffs for piglets) 0.005 (complementary feedingstuffs for piglets)	/	/	/
deoxynivalenol	/	12 (feed materials: maize by-products) 8 (feed materials: cereals and cereal products) 0.9 (complementary and complete feedingstuffs for pigs)	0.7 [3] (chronic effects)	2.8 [3] (acute effects)
zearalenone	/	3 (feed materials: maize by-products) 2 (feed materials: cereals and cereal products) 0.1 (complementary and complete feedingstuffs for piglets and gilts) 0.25 (complementary and complete feedingstuffs for sows and fattening pigs)	0.22 [4] (NOEL; female prepubertal piglets) 1 [4] (mature female pigs)	0.42 [4] (LOEL; female prepubertal piglets) 5 [4] (mature female pigs)
ochratoxin A	/	0.25 (feed materials: cereals and cereal products) 0.05 (complementary and complete feedingstuffs for pigs)	0.2 [5] (LOEL)	/
fumonisin B_1 and B_2	/	60 (feed materials: maize and maize products) 5 (complementary and complete feedingstuffs for pigs)	1 [6] (all fumonisins)	5 [6] (all fumonisins)

[1] Directive 2002/32/EC [13], [2] Commission Recommendation 2006/576/EC [14], [3] European Food Safety Authority (EFSA) [15], [4] EFSA [18], [5] EFSA [17], [6] EFSA [16].

In addition, the carry-over of mycotoxins into animal products for human consumption must be considered. Aflatoxins are of concern as a residue in milk, and pig meat can be an important source of ochratoxin A in human diets, while carry-over of DON and ZEN is insignificant [9]. The accumulation of fumonisins in edible tissues is considered to be low except for the liver and kidneys [19].

In 2004, the EFSA [20] warned about roughage and bedding being contaminated with ZEN, and thus contributing to the overall exposure of the animals. However, three years later, the EFSA evaluated the risk of mycotoxin contamination of fresh and adequately stored organic enrichment material as low [21]. The risk of mycotoxins posed by organic materials used as enrichment material in pig farming is discussed controversially, and therefore, this study aimed to evaluate the level of mycotoxin contamination of different organic enrichment materials to assess the potential health risks for pigs.

2. Materials and Methods

To represent the use of diverse materials as enrichment for pigs, 21 different organic materials commonly used as enrichment materials were selected for this study. These materials included four wooden materials, seven materials made of loose straw or hay, four materials consisting of compressed straw and hay, and the remaining six materials are summed up as miscellaneous group because of the wide differences of raw materials within this group. The analyzed organic materials and their respective groups are shown in Table 2.

Table 2. Organic enrichment materials considered in the study.

Material
Wooden materials
Wood granulate
Wood shavings
Sawdust
Millings
Loose straw and hay
Flax (*Linum* L.) straw
Wheat (*Triticum* L.), rye (*Secale cereal* L.), triticale (*Triticale* Tscherm.-Seys. ex Müntzing) straw meal
Alfalfa (*Medicago sativa* L.) hay mixed with green harvested oats (*Avena* L.) and clover (*Trifolium* L.) and treated with molasses and vegetable oil
Rye (*Secale cereal* L.) straw meal
Hemp (*Cannabis* L.) straw
Grass hay (from farm)
Wheat (*Triticum* L.) straw (from farm)
Compressed straw and hay
Compressed wheat (*Triticum* L.) and rape (*Brassica napus* L.) straw cylinder
Wheat (*Triticum* L.) pellets
Grass and herb hay pellets
Miscanthus (*Miscanthus* x *giganteus*J.M.GREEF & DEUTER EX HODK. & RENVOIZE) cylinder
Miscellaneous
Sugar beet (*Beta vulgaris* subsp. *vulgaris* L.) pulp with molasses
Maize (*Zea mays* L.) pellets
Peat (rooting material for piglets)
Lick block made of dehydrated molasses, vegetable fat, and mineral nutrients
Lignocellulose litter
Maize (*Zea mays* L.) silage (from farm)

The materials were commercially available except for wheat straw, hay, and maize silage, which were produced on the Farm for Education and Research in Ruthe of the University of Veterinary Medicine Hannover, Foundation. Wagner et al. [22] published on the hygienic status of these materials especially regarding the occurrence of bacteria and molds. A first batch of ten samples were examined in July 2015: wood granulate; straw meal made of wheat, rye, and triticale; alfalfa hay mix; straw meal made of rye; wheat straw from the university farm; hay pellets; maize pellets; peat; lick block; lignocellulose; maize silage. Samples of the remaining eleven materials were taken in May 2017 from subsequently ordered batches of material. The hay, straw, and maize silage from farms were sampled by taking ten subsamples of approximately 100 g of ten different locations (maize silage) or ten different bales (hay and straw). The commercially available materials were opened with cleaned and disinfected scissors. Subsequently, subsamples of ten different locations within each material batch were taken. Depending on the batch size, each subsample weighed 50–100 g. Concerning the wheat straw and miscanthus cylinders, parts of ten different cylinders were included in the sample. Each material sample was transferred into sterile plastic bags and sent to an external laboratory. One lick block was sent in whole and unopened to the laboratory, where it was further processed. The materials were analyzed for mycotoxins in a specialized laboratory (IFA Tulln, Austria), as described before [23]. Briefly, the samples were ground, and 5 g of each sample were extracted with 20 mL of extraction solvent consisting of acetonitrile, water, and acetic acid (79:20:1, *v*/*v*/*v*). The extraction was conducted in a rotary shaker for 90 min with subsequent centrifugation. After a dilution step (acetonitrile/water/acetic acid 20:79:1, *v*/*v*/*v*), 50 µL of the extracted sample were analyzed by a liquid chromatography tandem mass spectrometry multi-mycotoxin method. The limits of detection for aflatoxin B_1, DON, ZEN, ochratoxin A, and fumonisin B_1 and B_2 were 1.5, 1.5, 0.3, 1.5, 4, and 4 µg/kg, respectively. The materials were tested for over 380 mycotoxins and metabolites, but this paper is focused on mycotoxins with maximum permissible values or guidance values given by the European Union: aflatoxin B_1, deoxynivalenol, zearalenone, ochratoxin A, and fumonisin B_1 and B_2 [13,14]. An overview of the results of all analyzed mycotoxins is shown in Tables S1–S21 in the Supplementary Materials. In addition, the dry matter (DM) content of each material was measured with a Sartorius MA40 Moisture Analyzer (Sartorius AG, Göttingen, Germany). For a better comparability to the values in the EU regulation, the results of the aforementioned mycotoxins are given in mg mycotoxin/kg organic material corrected to 88% DM content. Data presentation and evaluation were performed descriptively with MS Excel 2010 (Microsoft Corporation, Redmond, Washington, USA) due to the limited number of tested materials and the differences in material characteristics.

3. Results

The DM content and the total number of detected mycotoxins in each material are shown in Table 3. For most materials, the DM content ranged between 85.61 (hay) and 98.58% (millings) except for peat and maize silage with a DM content of 31.83 and 27.13%, respectively. None of the tested organic materials were free of mycotoxins. They contained between four (wood shavings) and 64 (maize pellets) different mycotoxins.

Aflatoxin B_1 and ochratoxin A were not detected in any of the tested materials. The only finding of fumonisins was fumonisin B_2 with a concentration of 0.014 mg/kg in straw meal made of wheat, rye, and triticale. All concentrations of DON and ZEN are shown in Tables 4 and 5, respectively. DON was detected in eight of the tested materials, and the concentrations varied considerably from 0.002 (lignocellulose litter) to 5.006 mg/kg (maize pellets).

Table 3. Dry matter content (%) and total number of detected mycotoxins in the tested materials.

Material	Dry Matter	Number of Mycotoxins
Wooden materials		
Wood granulate	85.80	29
Wood shavings	86.81	4
Sawdust	89.79	10
Millings	98.58	9
Loose straw and hay		
Flax straw	85.70	23
Wheat, rye, triticale straw meal	87.91	52
Alfalfa hay	88.45	38
Rye straw meal	88.29	41
Hemp straw	86.85	23
Hay (from farm)	85.61	41
Wheat straw (from farm)	86.78	54
Compressed straw and hay		
Compressed straw cylinder	89.17	29
Straw pellets	90.26	52
Hay pellets	90.92	57
Miscanthus cylinder	91.00	35
Miscellaneous		
Beet pulp with molasses	93.76	17
Maize pellets	93.56	64
Peat	31.83	16
Lick block	95.00 [1]	22
Lignocellulose	88.48	34
Maize silage	27.13	56

[1] According to the manufacturer.

Table 4. Concentration of deoxynivalenol (DON) in the tested organic materials in mg mycotoxin/kg material based on a dry matter content of 88%.

Material	DON [1]
Wooden materials	
Wood granulate	<LOD
Wood shavings	<LOD
Sawdust	<LOD
Millings	<LOD
Loose straw and hay	
Flax straw	<LOD
Wheat, rye, triticale straw meal	0.503
Alfalfa hay	0.015
Rye straw meal	0.202
Hemp straw	<LOD
Hay (from farm)	<LOD
Wheat straw (from farm)	0.137
Compressed straw and hay	
Compressed straw cylinder	0.036
Straw pellets	<LOD
Hay pellets	<LOD
Miscanthus cylinder	<LOD
Miscellaneous	
Beet pulp with molasses	<LOD
Maize pellets	5.006
Peat	<LOD
Lick block	<LOD
Lignocellulose	0.002
Maize silage	2.118

[1] LOD = limit of detection.

Table 5. Concentration of zearalenone (ZEN) in the tested organic materials in mg mycotoxin/kg material, based on a dry matter content of 88%.

Material	ZEN [1]
Wooden materials	
Wood granulate	<LOD
Wood shavings	<LOD
Sawdust	<LOD
Millings	<LOD
Loose straw and hay	
Flax straw	<LOD
Wheat, rye, triticale straw meal	0.031
Alfalfa hay	0.0004
Rye straw meal	<LOD
Hemp straw	0.010
Hay (from farm)	0.300
Wheat straw (from farm)	0.033
Compressed straw and hay	
Compressed straw cylinder	<LOD
Straw pellets	0.002
Hay pellets	<LOD
Miscanthus cylinder	0.011
Miscellaneous	
Beet pulp with molasses	0.002
Maize pellets	1.208
Peat	<LOD
Lick block	0.0002
Lignocellulose	<LOD
Maize silage	0.254

[1] LOD = limit of detection.

The detected concentration of ZEN ranged from 0.0002 mg/kg in the lick block to 1.208 mg/kg in the maize pellets.

4. Discussion

Mycotoxins are secondary metabolites of filamentous fungi [24]. Of the several thousand existing secondary metabolites, only a minority has already been tested for their toxicity [9]. To date, approximately 400 secondary metabolites have been identified as being toxic to animals, i.e., as mycotoxins [24]. Due to their effects on animal health and their prevalence on farms, the following discussion is focused on mycotoxins considered in the EU regulation with maximum permissible or guidance values [13,14].

Chromatography combined with mass spectrometry is recommended by the European Commission [25] for mycotoxin detection in feed. Liquid chromatography coupled with tandem mass spectrometry is by far the most common method in the recent scientific literature [26]. However, mycotoxin contamination is highly variable between different harvest seasons and regions, because the growth of fungi and the formation of mycotoxins depends on several factors, primarily climatic and weather conditions [9]. Furthermore, the heterogenous distribution of mycotoxins with highly contaminated "hot-spots" exacerbates robust sampling [27]. Hay and straw harvested on farms are the most commonly used enrichment material in pig husbandry, but those materials differ greatly in their quality from farm to farm and are not comparable. To represent these materials, hay, straw, and maize silage were obtained from one example farm. The detected mycotoxin contaminations are only intended to be a preliminary guide of what levels can be expected and cannot be extrapolated to materials from other farms or material harvested at other points in time. Since the other materials were commercially available, more comparability is expected due to standard processing steps and quality control measures. However, the analysis was limited to 21 materials with substantial differences in the raw materials and material characteristics. Thus, the results of this study can only give indications as to which materials potentially pose risks and therefore must be monitored closely.

Aflatoxin B_1 is the most common and most toxic aflatoxin in agricultural products [28]. It typically occurs in products from tropical and subtropical regions but also in Europe, for example in maize [28]. Aflatoxins are mycotoxins predominantly produced by *Aspergillus flavus* and *Aspergillus parasiticus* [12]. They are hepatotoxic, carcinogenic, and immunotoxic [29]. The immunotoxicity can lead to failures of vaccination and reduced resilience against infections in farm animals [12]. In pigs, intoxication with low levels of aflatoxins impairs growth performance, especially in combination with other mycotoxins such as DON [30]. However, acute aflatoxicosis with severe clinical signs and increased mortality has also been described [31]. Aflatoxin B_1 was not detected in any of the tested enrichment materials in this study. Therefore, we assume that the tested enrichment materials pose no risk of aflatoxin intoxication to swine.

The trichothecene-mycotoxin deoxynivalenol (DON) is produced pre-harvest primarily by *Fusarium graminearum* and *Fusarium culmorum* [32]. It is common in cereals such as wheat, triticale, and maize grains [32]. The most important acute adverse health effect of DON in pigs is vomiting [33]; chronic adverse effects include reduced feed intake and reduced body weight gain [34]. DON is frequently detected in maize [10], and thus, the highest recommended guidance value in Commission Recommendation 2006/576/EC [14] exists for maize by-products (12 mg/kg). The recommendations for cereals and cereal products is 8 mg/kg [14]. However, swine have high sensitivity, and the guidance value for pig feed is only 0.9 mg DON/kg feed [14]. More than half of the tested organic enrichment materials were free of detectable DON concentrations, whereas the two maize products (pellets and silage) showed high levels of DON contamination (5.0 and 2.1 mg/kg, respectively). The level of DON in the maize pellets and maize silage exceeded the guidance value for pig feed by a factor of over five and over two, respectively. As LOAEL for vomiting, the EFSA identified a concentration of 2.8 mg DON/kg feed, while the LOAEL for chronic adverse effects was difficult to determine due to a range of 0.35–13 mg DON/kg feed described in the literature [15]. Based on these levels, only the maize pellets pose an acute health risk, whereas both maize pellets and maize silage—as well as straw meal made of wheat, rye, and triticale with 0.5 mg DON/kg—might compromise the health of pigs when given over a longer period of time.

The detected high levels of DON are in agreement with previously reported mycotoxin contamination. In a Swedish study [7], the average level of DON in straw samples was 0.9 mg/kg, and the median 0.4 mg/kg. The level of 2.1 mg DON/kg in maize silage in this study exceeded average levels of 0.8 mg/kg (0.7 mg/kg corrected to 88% DM content) [35] and 0.7 mg/kg (0.6 mg/kg corrected to 88% DM content) [36] but was below the reported maximum levels of 3.0 mg/kg (2.6 mg/kg corrected to 88% DM content) and 3.1 mg/kg (2.7 mg/kg corrected to 88% DM content). The maize pellets are difficult to compare to other studies, because they are made of the whole maize plants and not only the maize kernels and are primarily marketed as horse feed. Thus, it is not a common material in mycotoxin studies. In a study about mycotoxins in horse feed [37], the DON contamination of maize-based feed ranged from 0.016 to 4.9 mg/kg. The present level of 5.0 mg/kg is a high level of DON contamination, but it is consistent with previous findings in similar materials.

Zearalenone (ZEN) is a mycotoxin produced by fungi of the genus *Fusarium* and a common contaminant particularly in wheat and maize [27]. The main adverse health effect of ZEN is due to its ability to bind estrogenic receptors and behave like endogenous 17-β-estradiol causing hyperestrogenism [38]. The toxicity of ZEN varies by species, age, sex, stage of estrus cycle, and presence or absence of pregnancy [8]. Pigs are a highly sensitive species particularly prepubertal female piglets [38]. Clinical signs of ZEN intoxication include vulvovaginitis, reduced fertility and litter size, as well as signs of hyperestrogenism in new-born piglets [39]. Furthermore, ZEN consumption can result in depressed serum testosterone, spermatogenesis, and libido in boars [39].

Similar to DON, the highest guidance values for ZEN are given for maize by-products (3 mg/kg) and cereal and cereal products (2 mg/kg) [14]. Considering the varying susceptibility of various species and ages, the Commission Recommendation [14] defines different guidance values for feed meant for sows and fattening pigs (0.25 mg/kg) versus for piglets and gilts (0.1 mg/kg). All detected amounts of ZEN in our study were below the guidance values for cereal and cereal products and maize by-products. However, the levels of ZEN found in the maize pellets (1.21 mg/kg) and hay (0.30 mg/kg) exceeded the guidance value for feed for sows and fattening pigs, while the level in maize silage (0.25 mg/kg) reached this guidance value. Thus, all three aforementioned materials showed a higher ZEN contamination than recommended for feed for piglets and gilts. The EFSA [18] identified 0.22 mg ZEN/kg feed as NOEL for piglets and 1 mg ZEN/kg feed as NOAEL in mature female pigs. Since the NOEL for piglets lies between the two guidance values of the EU Commission, the maize pellets, hay, and maize silage all exceeded the NOEL, and health effects have to be expected when those materials are given to piglets. The marked contamination of the maize pellets even exceeded the NOAEL for mature pigs; thus, this material should not be used in pig husbandry. Considering ZEN, the materials examined in this study might pose a health risk for pigs and should be considered in combination with the ZEN levels of the feed.

The detected amounts of ZEN in maize pellets and hay are elevated compared to levels reported before. ZEN contamination in maize-based horse feed reached a maximum level of 0.3 mg/kg [37], which is only a quarter of the contamination of the maize pellets. For hay, levels of up to 0.1 mg/kg were detected in a Czech study [35]. However, as mentioned before, the maize pellets are not exactly the same type of material as analyzed in the horse feed study, and in the Czech study, only four hay samples were analyzed. The ZEN contamination of maize silage was higher than reported in the Czech study (maximum 0.1 mg/kg) [35], but in accordance with findings in the Netherlands (average of positive samples 0.2 mg/kg; maximum 0.8 mg/kg) [36].

Fumonisins are also mainly produced by *Fusarium* spp. and are frequently found in cereal grains and especially in maize [40]. Fumonisin B_1 is the most toxic fumonisin and causes fatal porcine pulmonary edema syndrome [9]. The target organs of fumonisin toxicity in pigs in general are the lung, liver, kidneys, and heart [19,37]. Pierron et al. [29] summarized studies showing the effect of fumonisins on the immune system including impairment of vaccination effectiveness. The guidance value given in Commission Recommendation 2006/576/EC [14] is a combined value for fumonisin B_1 and B_2 and is 60 mg/kg for maize and maize products and 5 mg/kg for pig feedingstuffs. A NOAEL and LOAEL of 1 and 5 mg fumonisin/kg feed, respectively, were determined for pigs by the EFSA [16]. Straw meal made of wheat, rye, and triticale was the only material in this study with a detectable amount of fumonisin contamination. However, the detected concentration of 0.014 mg fumonisin B_2/kg was considerably lower than any given guidance value, NOAEL and LOAEL. Polarity and matrix effects could possibly lead to reduced recovery rates of fumonisins in multitoxin methods [41]. Thus, the detected levels of fumonisins might be underestimated in this study. However, we consider this deficit in the method to be negligible, since the NOAEL is 70-fold higher than the only finding of fumonisin. We conclude that the tested enrichment materials do not pose a risk for pigs concerning fumonisins.

Ochratoxin A is produced mainly because of inadequate post-harvest storage; the fungal source of the toxin varies depending on the climatic conditions [42]. In warm regions, *Aspergillus ochraceus* is the main producer, and *Penicillium verrucosum* is responsible for contamination with ochratoxin A in colder regions [42]. Ochratoxin A is a very common contaminant in South Asia but also occurs in many European feed samples [10]. The toxicity of ochratoxin A primarily affects the kidneys but also the liver and immune system [29]. Pigs are particularly sensitive to these toxic effects, which can lead to acute outbreaks as well as cases of chronic intoxication [8]. These both become clinical by mycotoxic porcine nephropathy [8]. Ochratoxin A was not detected in any of the tested materials in this study. Therefore, they all comply with the guidance values set by the EU [14] and pose no risk for ochratoxin A intoxication.

The latest study comprising the most extensive dataset of mycotoxin contamination in feed [10] showed that maize has a high prevalence of mycotoxin contamination. Generally, co-contamination with different mycotoxins is common, and the concentrations of DON and ZEN are positively correlated [10]. This is partly reflected by the data of our study. The highest level of both DON and ZEN was found in the maize pellets. The maize silage also exceeded the EU guidance values for pigs in both mycotoxins. However, the second highest level of ZEN was detected in grass hay produced on a farm, which did not contain a detectable amount of DON. The relatively low prevalence of aflatoxin B_1 and ochratoxin A of 12.7 and 11.9% in Central Europe was described by Gruber-Dorninger et al. [10] and supported our results, because neither mycotoxin was detected in this study. However, the formerly detected high prevalence and high level of contamination with fumonisins in feed, especially in maize [10], is not in agreement with our results. Only 21 organic materials were tested here and not all of them were feed or feed raw materials; thus, these results do not contradict the previous findings.

As mentioned before, moisture is a known risk factor for mold growth and mycotoxin formation. According to Bryden [9], moisture levels of 13–18% are sufficient for post-harvest mold growth. On the contrary, the DM was not a crucial factor related to mycotoxin burden in this study. The two materials with by far the lowest DM content, peat (31.83%) and maize silage (27.13%), differed in their mycotoxin contamination considerably. In peat, the total number of detected mycotoxins was only 16, and none of the main mycotoxins discussed in this paper were detected. Maize silage was contaminated with 56 different mycotoxins and exceeded guidance values of both DON and ZEN. Presumably, in this case, the large differences in the raw materials were the most important factor. Comparing the highest and lowest DM content in the loose straw and hay group, namely alfalfa hay (88.45%) and hay from farm (85.61%), the differences in the total number of mycotoxins (38 and 41, respectively) and DON concentration (0.015 mg/kg and no detection, respectively) were minor. In contrast, the level of ZEN (0.0004 and 0.300 mg/kg, respectively) varied considerably. However, as field fungi, *Fusarium* spp. produce mycotoxins pre-harvest [8]. Thus, the detection of *Fusarium* toxins is only a retrospective indicator for the conditions on field and is not directly related to the DM content during storage. Aflatoxin B_1 and ochratoxin A are commonly produced post-harvest [8]. However, neither of these mycotoxins were detected here, and we cannot correlate their concentration to the DM content of the tested materials.

Enrichment materials are provided and ingested in considerably smaller amounts than feed, and the maximum permissible and guidance values cannot be adopted without limitations. However, recommendations on the minimal amount of enrichment material per day have not been established [2,6]. Sows may use up to 500 g of straw per day if given the opportunity, and pigs were willing to work for up to 1 kg of straw [2]. On the other hand, weaned pigs, when given straw ad libitum in a straw rack, consumed on average as little as 5 g per pig per day, which still had a positive effect on tail wounds [43]. Most studies discuss different amounts of straw, while comparative data for other materials are lacking. In addition, one can presume that there are individual differences in the intake of enrichment materials that are influenced by different factors. This relativizes the risk for mycotoxin intoxication posed by organic enrichment materials and shows that it is impossible to calculate this risk precisely. The EFSA [18] identified 17.6 µg ZEN/kg bodyweight per day as LOEL for piglets and 200 µg ZEN/kg bodyweight per day as LOAEL in mature female pigs. For mature pigs, the LOAEL realistically cannot be reached by consumption of the contaminated enrichment materials, but they have to be considered as additive source of ZEN to the potential contamination existent in feed. For piglets, however, the LOEL might be reached or even exceeded by the tested materials. If, for example, a piglet with a bodyweight of 10 kg is provided with the maize pellets (1.208 mg ZEN/kg), the level is reached by merely 145.7 g (if not corrected to 88% DM: 155.2 g) of the pellets. However, if the feed is also contaminated with ZEN, the level of health effects is reached with the consumption of even less enrichment material, which might be a realistic value of daily intake for piglets. However, besides the obvious adverse health effects, mycotoxin ingestion leads to various alterations on productivity, resilience, and reproduction representing potentially major economic losses for farmers [42].

Cereal grains especially maize are also major components of pig nutrition, and thus, any risk assessment must consider the combined mycotoxin level of feed and enrichment materials. Some of the enrichment materials are also feed or feed raw materials, and thus, harvest years with weather conditions leading to high mycotoxin values will probably affect both the pig feed and the enrichment material. In those cases, materials with low risks of mycotoxin contamination, such as wooden materials, should be used as enrichment. The use of maize products as enrichment material for pigs cannot be recommended due to the high risk of considerable mycotoxin contamination in these materials.

5. Conclusions

DON and ZEN contamination in enrichment material depends on the source organic material. It can exceed the guidance values given by the EU as well as the lowest-observed-adverse-effect levels (LOAEL) provided by the EFSA leading to swine health risks. In this study, maize products showed particularly high levels of mycotoxins. However, it is not possible to assess the exact risk to date, and further studies are needed, because the amount of enrichment material consumed by pigs of different ages is still unknown. These findings do not contradict the use of enrichment material to fulfil the behavioral needs of the pigs, but these materials should be sourced based on the same criteria as feed in terms of hygiene and quality.

Supplementary Materials: The following materials are available online at http://www.mdpi.com/2077-0472/10/11/565/s1: Table S1: Detected mycotoxins and other metabolites in wood granulate in µg mycotoxin/kg material; Table S2: Detected mycotoxins and other metabolites in wood shavings in µg mycotoxin/kg material; Table S3: Detected mycotoxins and other metabolites in sawdust in µg mycotoxin/kg material; Table S4: Detected mycotoxins and other metabolites in millings in µg mycotoxin/kg material; Table S5: Detected mycotoxins and other metabolites in flax straw in µg mycotoxin/kg material; Table S6: Detected mycotoxins and other metabolites in wheat, rye, triticale straw meal in µg mycotoxin/kg material; Table S7: Detected mycotoxins and other metabolites in alfalfa hay in µg mycotoxin/kg material; Table S8: Detected mycotoxins and other metabolites in rye straw in µg mycotoxin/kg material; Table S9: Detected mycotoxins and other metabolites in hemp straw in µg mycotoxin/kg material; Table S10: Detected mycotoxins and other metabolites in hay (from farm) in µg mycotoxin/kg material; Table S11: Detected mycotoxins and other metabolites in wheat straw (from farm) in µg mycotoxin/kg material; Table S12: Detected mycotoxins and other metabolites in compressed straw cylinder in µg mycotoxin/kg material; Table S13: Detected mycotoxins and other metabolites in straw pellets in µg mycotoxin/kg material; Table S14: Detected mycotoxins and other metabolites in hay pellets in µg mycotoxin/kg material; Table S15: Detected mycotoxins and other metabolites in miscanthus cylinder in µg mycotoxin/kg material; Table S16: Detected mycotoxins and other metabolites in beet pulp with molasses in µg mycotoxin/kg material; Table S17: Detected mycotoxins and other metabolites in maize pellets in µg mycotoxin/kg material; Table S18: Detected mycotoxins and other metabolites in peat in µg mycotoxin/kg material; Table S19: Detected mycotoxins and other metabolites in lick block in µg mycotoxin/kg material; Table S20: Detected mycotoxins and other metabolites in lignocellulose in µg mycotoxin/kg material; and Table S21: Detected mycotoxins and other metabolites in maize silage in µg mycotoxin/kg material

Author Contributions: Conceptualization, N.K. and J.S.; methodology, N.K. and J.S.; validation, K.M.T. and J.S.; formal analysis, K.M.T.; investigation, K.M.T.; resources, N.K.; data curation, K.M.T.; writing—original draft preparation, K.M.T.; writing—review and editing, N.K.; supervision, N.K.; project administration, J.S. and N.K.; funding acquisition, N.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the Tierseuchenkasse Niedersachsen (Animal Disease Fund Lower Saxony, Germany). K.M.T. was kindly financially supported by a grant from the H. Wilhelm Schaumann Stiftung.

Acknowledgments: We thank Wolfgang Markert and Thomas Erhäusl BIOMIN GmbH for mycotoxin analysis at IFA, Tulln.

Conflicts of Interest: The authors declare no conflict of interest.

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