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Fusarium mycotoxin contamination of cereals and bedding straw at Swedish pig farms

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ABSTRACT

Fusarium mycotoxins such as DON and ZEN are commonly occurring in both cereal grains and straw particularly in temperate regions. Pigs are among the most sensitive animals to exposure of these mycotoxins. Straw is the most commonly used bedding material and thus may give a significant contribution to the exposure to pigs. The objective of the present study was to screen cereals and bedding material (straw) produced at Swedish pig farms for the presence of DON, ZEN, T-2 and HT-2 toxins. 188 samples from 2011 and 2012 were sampled and analysed, where DON was almost ubiquitous with 89% of the samples being contaminated. ZEN was present in 54% of the samples while T-2 and HT-2-toxin was detected in 29 and 16%, respectively. Oats was the cereal grain most frequently contaminated with DON and ZEN. Eight out of ten samples with highest ZEN levels were from straw whereas five out of ten samples of oat kernels contained the highest DON levels. Four out of ten samples were contaminated with both high levels of DON and ZEN. The large variation in DON and ZEN levels in the straw and cereals between the different farms yielded a large difference in the animal exposures at the studied farms.

1. Introduction

The mould genus *Fusarium* is an important cereal pathogen worldwide and it is well known that several *Fusarium* species may produce toxic secondary metabolites (mycotoxins) (van der Fels-Klerx et al., 2012). Following infection of the cereal plant mycotoxins may accumulate, resulting in contamination of animal feed and human cereal food in toxicologically relevant concentrations (Rodrigues & Naehrer, 2012). The most important mycotoxins in cereals in Northern Europe are most likely the trichothecenes, a large group of structurally related toxic compounds, as well as zearalenone (ZEN).

In vitro studies have demonstrated a multitude of toxic effects on eukaryotic cells by trichothecenes, very importantly effects on the protein synthesis as well as cytotoxic and effects on apoptosis (Pinton et al., 2012). In animal studies several toxic effects have been demonstrated as well as historical cases of field disease outbreaks most likely caused by *Fusarium* mycotoxins (Morgavi & Riley, 2007).

Acute toxicosis results in a shock-like response including diarrhea, vomiting, leucocytosis and hemorrhage also including mortality at higher doses (Pestka, 2007). Chronic exposure can lead to anorexia, reduced weight gain as well as nutritional efficiency, neuroendocrine changes and immune modulations (Pestka, 2008). In situations with natural occurrence it is

Abbreviations: DON, deoxynivalenol; ZEN, zearalenone; LOQ, Limit of detection (LOD) and 9 limit of quantification.

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commonly observed that the toxicity is higher than expected from its content of analysed mycotoxins. In the last decade information has become available that mycotoxins may also occur in conjugated forms or associated with macromolecules (Berthiller et al., 2013). This new data is presently being discussed by scientists and legislators in relation to safety aspects of feed and food.

One of the most commonly detected trichothecenes in cereals is 4-deoxynivalenol (DON), a mycotoxin to which pigs seem to be the most susceptible species among the domestic animals (Pestka, 2008). The absorption is rapid and already 4 h after consumption maximum serum concentrations are reached (Pestka, 2007). In contrast to ruminants, de-epoxidation of DON by the gut microflora in pigs is of less importance, resulting in a higher proportion of DON being absorbed from the feed (Dänicke et al., 2004).

Another *Fusarium* mycotoxin of toxicological significance for pigs is zearalenone (ZEN). ZEN is a non-steroidal oestrogenic mycotoxin synthesized through a polyketide pathway by several *Fusarium* species, commonly being soil fungi infecting cereal crops worldwide (Tiemann & Dänicke, 2007).

The presence and often co-occurrence of DON and ZEN in cereal pig feed has emerged as a severe health, welfare and reproduction problem worldwide because of multitude of effects by the mycotoxins, also affecting the economy of the farmer (D'Mello et al., 1999, Pestka, 2007).

In recent surveys it was shown that cereals used in European pig feed are commonly contaminated with DON and/or ZEN as well as other trichothecenes such as T-2 and HT-2 toxins (Streit et al., 2012, EFSA, 2013). It was also demonstrated that the bioavailability of DON from wheat straw was extensive and may contribute to the exposure to *Fusarium* mycotoxins in pigs following ingestion (Rohweder et al., 2013).

For animal welfare reasons straw is commonly used as bedding material in pig production and the present knowledge about mycotoxin exposure from straw, compared to feed, is limited. Interestingly, one study suggests that DON exposure via contaminated bedding straw was the cause of sudden weight loss in horses (as reported in Newman and Raymond, 2005).

Swedish pig farming is characterized by individual farms usually with associated farm land where most of the cereals used as feed and bedding straw is produced. From a mycotoxin point of view the use of cereals and straw produced on the farm may strongly influence the exposure because of large variations in toxin levels between individual farms and limited trade.

The Swedish animal welfare legislation states that pigs must have access to bedding material (usually straw) in adequate amounts for carrying out their natural behavior (SJVFS, 2010:15).

The aim of the present investigation was to screen cereals and straw harvested and stored at pig farms in Sweden in 2011 and 2012 for the presence of the common *Fusarium* mycotoxins, in order to reveal the distribution and levels of these mycotoxins under field conditions primarily to be able to assess possible effects on pig health and production.

2. Materials and methods

Samples of cereals and straw, produced on the farm, from the harvest of 2011 and 2012, respectively, were collected between September and January by veterinarians from the Swedish Animal Health Service. The instructions to each veterinarian were to collect samples at two pig farms of all accessible lots of dried cereal grain and straw intended for the pig production and send the samples to the National Veterinary Institute. Lot being defined as grain stored in one specific place and described by species or straw from one species and harvest time stored at one place.

Sampling of cereals and straw was carried out by taking 10–20 incremental samples from various parts of the lot. The increments were mixed into one laboratory sample consisting of 0.5–1 kg.

The geographical distribution of the samples, also representing the regions where a vast majority of the Swedish pig production is located, is illustrated in Fig. 1. All samples were dried under weight control, at 60 degrees C overnight (16 h) in a ventilated drying cupboard prior to grinding on a hammer mill (Falling Number/Perten Instruments) to pass a 1 mm screen. The water-content of the grain and straw was estimated by assuming, on average, a rest water content of 7 g/100 g after drying and milling.

Analyses of DON, ZEN, T-2 and HT-2 toxins were performed within a few weeks after grinding using LC-MS/MS according to Tevell Åberg et al. (2013). Limits of detection (LOD) and limits of quantification (LOQ) were for DON 50 and 300 μg/kg, for ZEN 2 and 30 μg/kg respectively, and for HT-2 and T-2 toxins 1 and 40 μg/kg, respectively. All results were reported on a dry matter basis.

The data was initially subjected to descriptive statistics (range, median and mean), however, since the toxin levels showed a skewed distributions (Fig. 2a–d), it was concluded that analyses by parametric methods relying on normality should be avoided. The results are therefore presented graphically and in tables.

3. Results

A total of 188 samples of cereal grain and straw were collected from 31 pig farms in 2011 and 27 farms in 2012 where nine farms were sampled both years. The water-content of the grain samples was on average 16% or 17%, respectively, for the straw. One straw sample had a water-content of 68% and was not included in the calculations.

In Fig. 1 the geographical distribution of cereals and straw sampled in 2011 and in 2012 is indicated. The maps also show the most important areas for pig farming in Sweden. Barley was the most often sampled feed grain followed by wheat, oats,

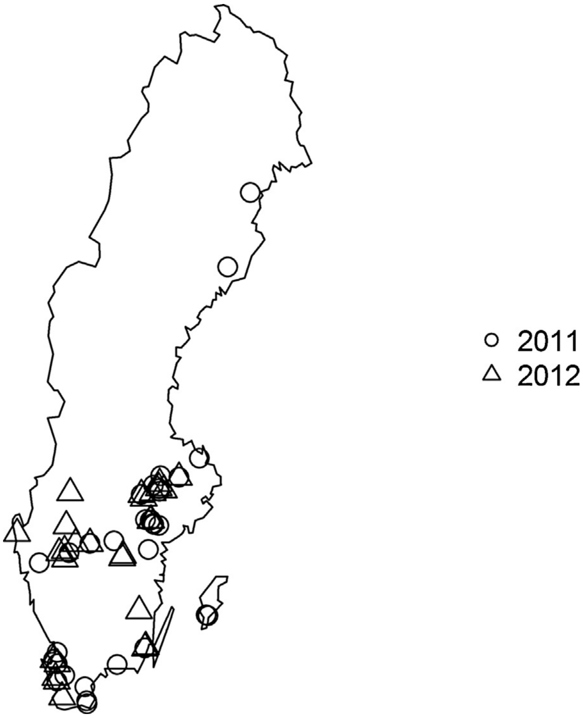

Fig. 1. Geographical distribution of cereals and straw sampled at pig farms in 2011 and 2012.

mixed grain and triticale (Table 1). Of the total number of samples in the survey straw constituted approximately 42%. The species of the collected straw samples were not identified.

The distribution of DON and ZEN-levels in the cereal and straw samples are shown in Fig. 2a and b and in Table 2. The distribution of DON and ZEN in the samples was skewed with median values lower than the mean. About 68% of the samples contained 600 μg DON/kg or less (Fig. 2a) while in some samples the concentrations were considerably higher with a maximum of 21613 μg/kg (Table 2).

The average concentration was highest for DON (865ug/kg) followed by ZEN (76 μg/kg), HT-2 (24 μg/kg) and T-2 toxin (17 μg/kg). The fraction of samples containing (above LOD) DON were 89%, followed by 48% for ZEN, 29% for HT-2 and 16% for T-2 toxin (Table 2).

For ZEN the concentrations were lower compared to the detected DON levels with approximately 83% of the samples at a maximum of 60 μg/kg while 16 samples were above 200 μg/kg with the highest concentration at 1771 μg/kg (Fig. 2b).

Most samples contained low T-2 or HT-2 toxin levels with a total of 13 samples above 60 μg/kg for T-2 toxin and 19 samples for HT-2 toxin, respectively (Fig. 2c and d).

DON-levels above the limit of detection were detected in 86-100% of the tested cereals while T-2 toxin, on the other hand, was detected in a smaller number of samples (Table 3). By introducing a threshold value for DON of 600 μg/kg and 100 μg/kg for ZEN, HT-2 and T-2 toxin, respectively, elevated levels of *Fusarium* mycotoxins were extracted. EU guidelines state 900 μg/kg as recommended maximum levels in pig diets, but negative effects of DON have been reported levels as low as 350 μg/kg (Drochner et al., 2004, Verstraete, 2013). Clearly, oats was the cereal grain most often contaminated with elevated levels of the four mycotoxins followed by straw (Table 4). For barley and wheat grain a smaller numbers of samples were above the threshold values.

In Table 5 the cereals or straw with the highest concentrations of DON from 2011 and 2012 are listed. Oats was the cereal where the highest DON concentrations were detected both in 2011 and 2012 followed by straw. Notably, 8 out of 10 samples with high concentrations of DON were from 2012 and 5 were from straw collected in either 2011 or 2012. For ZEN 8 out of 10 samples with the highest concentrations were from straw (Table 6) and most samples with high ZEN concentrations were from 2012.

Table 1
Number of samples of cereal straw and cereal grain collected from pig farms.

Year	Straw	Oats	Barley	Wheat	Mixed grain	Triticale
2011 (*N* = 94)	39	14	21	16	3	1
2012 (*N* = 94)	40	13	20	13	8	0
Total (*N* = 188)	79	27	41	29	11	1

Table 2
Summary statistics of *Fusarium* mycotoxin content in cereals and straw.

		DON	ZEA	HT2	T2
Straw (*N* = 79)					
	Range (μg/kg DM)	<50–11195	<2–1771	<1–201	<1–87
	Average (μg/kg DM)	884	134	14	3
	Median (μg/kg DM)	365	12	0	0
	Number of samples containing toxins	68	46	14	4
Oats (*N* = 27)					
	Range (μg/kg DM)	<50–21613	<2–1030	<1–1224	<1–647
	Average (μg/kgDM)	2123	77	92	89
	Median (μg/kg DM)	461	23	12	14
	Number of samples containing toxins	26	16	16	16
Barley (*N* = 41)					
	Range (μg/kg DM)	<50–1729	<2–449	<1–153	<1–171
	Average (μg/kg DM)	321	20	20	13
	Median (μg/kg DM)	162	0	0	0
	Number of samples containing toxins	35	12	17	9
Wheat (*N* = 29)					
	Range (μg/kg DM)	<50–3230	<2–116	<1–13	<1–12
	Average (μg/kg DM)	551	17	1	0
	Median (μg/kg DM)	195	0	0	0
	Number of samples containing toxins	26	10	3	2
Mixed grain (*N* = 11)					
	Range (μg/kg DM)	<50–969	<2–175	<1–90	<1–<1
	Average (μg/kg DM)	202	25	14	0
	Median (μg/kg DM)	101	0	0	0
	Number of samples containing toxins	11	5	5	0
Triticale (*N* = 1)					
	Range (μg/kg DM)	3503	33	<1	<1
	Average (μg/kg DM)	n/a	n/a	n/a	n/a
	Median (μg/kg DM)	n/a	n/a	n/a	n/a
	Number of samples containing toxins	1	1		

Table 3
Relative degree (percentages) of contamination by *Fusarium* mycotoxins.

	N	DON	ZEN	HT-2	T-2
Limit of detection (μg/kg DM)		50	2	1	1
Straw	79	86	58	18	5
Oats	27	96	59	59	59
Barley	41	88	29	41	22
Wheat	29	90	34	10	7
Mixed grain	11	100	45	45	0
Triticale	1	100	100	0	0

Table 4
Fraction of samples (percentages) with elevated levels of *Fusarium* mycotoxins.

	DON	ZEN	HT2	T2
Threshold value (μg/kg DM)	600	100	100	100
Straw	33	20	5	0
Oats	48	11	15	22
Barley	20	5	5	5
Wheat	28	7	0	0
Mixed grain	9	9	0	0
Triticale	100	0	0	0

Table 5
Cereal and straw samples with the highest concentrations of DON.

Year	Material	DON (μg/kg DM)
2012	Oats	21613
2012	Straw	11195
2012	Straw	10414
2012	Oats	8118
2012	Oats	5678
2011	Oats	4951
2011	Straw	4595
2012	Oats	4094
2012	Straw	3632
2012	Straw	3530

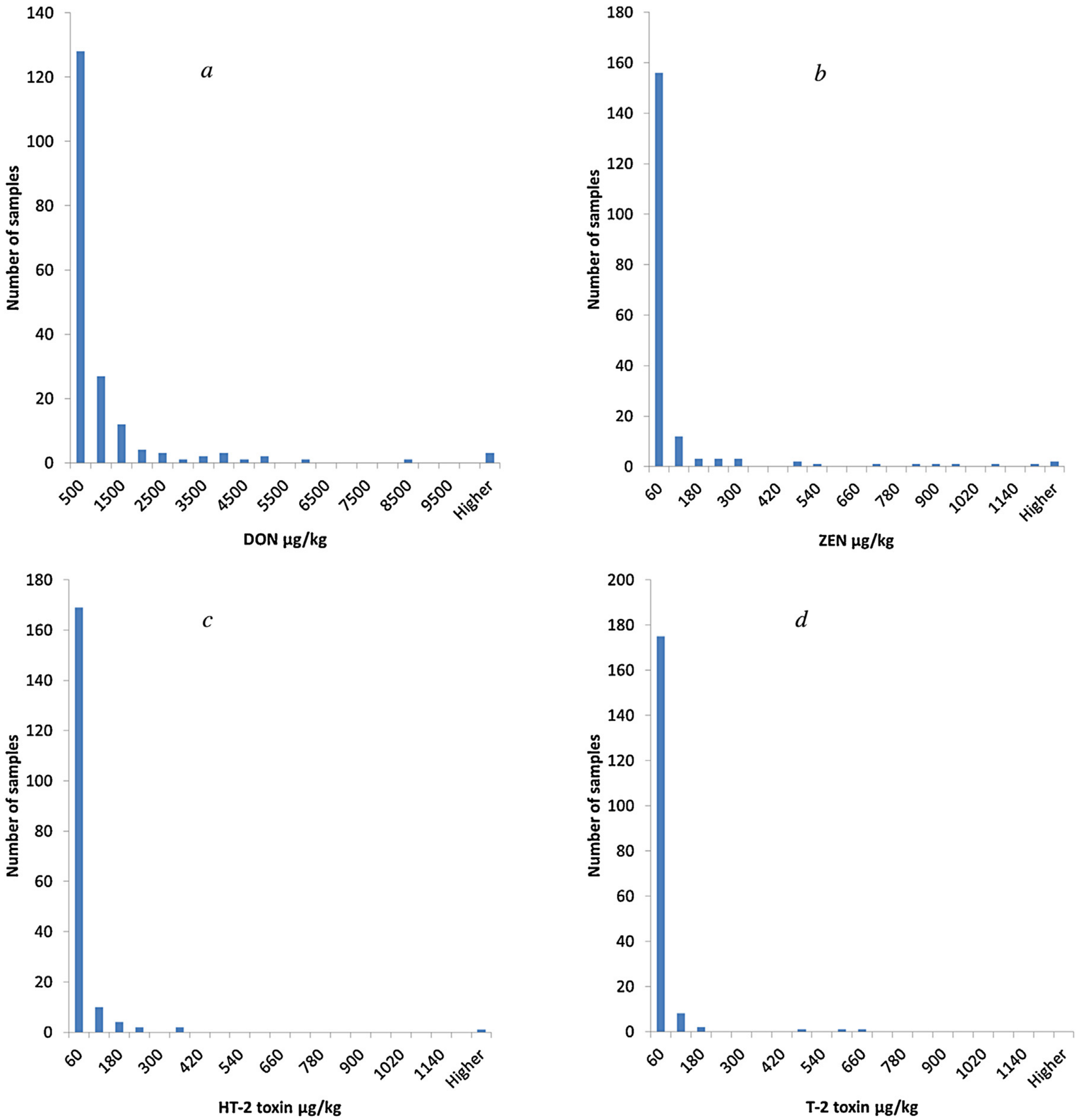

Fig. 2. a–d. Histograms showing the distribution of DON, ZEN, HT-2 and T-2 concentrations in cereals and straw samples from pig farms.

Table 6
Cereal and straw samples with the highest concentrations of ZEN.

Year	Material	ZEN (µg/kg DM)
2011	Straw	1771
2012	Straw	1400
2012	Straw	1170
2012	Oats	1030
2012	Straw	917
2012	Straw	897
2012	Straw	801
2011	Straw	664
2012	Straw	483
2011	Barley grain	449

Cereal samples from a few farms indicated elevated levels of both DON and ZEN. Of the ten highest DON and ZEN concentrations (Tables 5 and 6) four samples coincided. In particular oat grain from one farm showed a DON-content of 21613 μg/kg as well as a ZEN-level of 1030 μg/kg. In addition one sample of spring wheat grain containing 3632 μg/kg DON also showed a ZEN content of 1170 μg/kg.

4. Discussion

The results showed that trichothecene and ZEN exposure to the pigs will occur at different levels from cereals and straw. Some differences in geographical distribution of the samples from 2012 compared to 2011 were observed (Fig. 1). In 2012 more farms at the western part of Sweden were sampled, which was due to expected elevated mycotoxin levels in this region. For this reason no direct comparisons of feed and straw contamination by mycotoxins between 2011 and 2012 can be made.

The DM-content of collected cereals and straw indicated moisture levels which did not support growth of toxigenic *Fusarium* species, thus infections and subsequent mycotoxin formation in the field is the most likely reason for the contamination. Recent reports have also demonstrated the occurrence of mycotoxigenic *Fusarium* species as well as common trichothecenes in Swedish oats and wheat grain from a similar time period supporting the findings in the present study (Fredlund et al., 2013, Lindblad et al., 2013).

The highest average concentration of mycotoxins was, as expected, for DON followed by ZEN, HT-2 and T-2 toxin. Similarly, the maximum concentrations of the mycotoxins followed the same distribution with the highest DON-value in oats (Table 2). The distributions of mycotoxins were skewed, as illustrated in Fig. 2a–d. This is not surprising for mycotoxins, where a majority of samples often have low to moderate levels of toxins while a few samples may be heavily contaminated. One effect is the difference between median and mean values, where the latter can be strongly influenced by extreme values and thus become a less adequate descriptor of the population. In Table 2 this effect is evident and most pronounced for ZEN, HT2- and T2-toxins with median values of zero (lower bound) in several cases, stating that more than half of all samples had levels below LOD. Thus regional data based on average levels of contamination does not predict the underlying distribution of mycotoxin levels in the individual field or farm.

For DON 89% of investigated samples contained the mycotoxin. In recently published work the responsible toxigenic *Fusarium* species were reported from oats and wheat grain (Fredlund et al., 2013; Lindblad et al., 2013). The results showed a widespread occurrence of toxigenic species in oats with higher levels of *Fusarium graminearum*, DON and ZEN in the western part of Sweden.

In the present study the median DON and ZEN concentrations of all grain samples were similar to the median concentrations found in oats (Fredlund et al., 2013). In samples of winter wheat grain from 2009–2011 the median DON levels were lower, compared to the present study, while the results from spring wheat grain showed similar levels (Lindblad et al., 2013).

The high incidence of DON and ZEN positive straw samples, some at high levels, is challenging because bedding material must be used according to Swedish animal welfare legislation (SJVFS, 2010-15). In pig farming, straw produced on the farm, is the most common bedding material used.

According to van Barneveld (2003) the mean intake of straw from wheat and barley in 20–30 kg pigs was estimated to 13–14% of the diet. The recently demonstrated bioavailability of DON from wheat straw, in the same magnitude as from the grain, shows that straw may considerably contribute to the exposure to *Fusarium* mycotoxins in pigs (Rohweder et al., 2013).

A more challenging exposure to mycotoxins may occur if both cereals and straw produced on the farm are contaminated. Indeed, data from 2011 and 2012 demonstrated that 50% of the samples with the highest levels of DON were samples of straw (Table 5). For ZEN 80% of samples with the highest levels of the mycotoxin in the same period were from straw (Table 6).

The average concentration of DON and ZEN in the cereals and straw do not suggest that animal health disorders due to those mycotoxins are likely to occur. However, at individual farms with elevated levels of mycotoxins the levels may be exceeded. In a normal pig diet about 70–80% of the ration is cereals and the data shows that feeding certain lots of grain will result in diets exceeding the present EU-guidance levels. As reported by some veterinarians carrying out the sample collection, observations by the farmers also verifies that typical symptoms of feed refusal as well as reproductive disorders were observed. Clearly, the elevated DON and ZEN levels detected in straw justify further investigations of the bedding material as a source of mycotoxin exposure in pigs. Presently, there is a need for practical and accurate sampling methods for straw to be able to secure a safe bedding material at the farms.

The reasons why high levels of DON and ZEN were detected in the straw should also be addressed. Brinkmeyer et al. (2006) detected high levels of DON and ZEN in wheat after induced infection of winter wheat. The highest levels of toxins were in the glumes, followed by spindles and straw, which may be consistent with the fact that infection originated in the flowers. In the present study straw was sampled at storage, and thus it can be concluded that no or only a small fraction of glumes were present after combine harvesting. It is notable that the high levels of DON and ZEN seen in this field study were higher than the levels found in Brinkmeyer's et al. (2006) study where active inoculation with *Fusarium culmorum* was used.

Another important issue with contaminated straw as bedding material is that straw not only is a part of the diet but also of the close environment of growing piglets and sows after weaning. Since the concentration of DON in dust has been showed to be more than 10-fold compared to wheat kernels (Sanders et al., 2013), additional exposure to pigs by dust cannot be ruled out.

5. Conclusions

Investigations of cereals and straw at Swedish pig farms for the common *Fusarium* mycotoxins DON and ZEN have demonstrated their occurrence in most of the samples. Fifty per cent of the samples with the highest levels of DON were samples of straw. For ZEN 80% of samples with the highest levels of the mycotoxin were from straw. Because of the extensive bioavailability of DON from wheat straw, straw may considerably contribute to the exposure to *Fusarium* mycotoxins in pigs despite a limited part of the diet.

Conflicts of Interest

There are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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