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The surveillance programme for mould and mycotoxins in feed materials and complete feed in Norway 2014

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The surveillance programme for mould and mycotoxins in feed materials and complete feed in Norway 2014

Aksel Bernhoft, Ellen Christensen, Hege Divon, Thor A. Waaler, Chiek Er, Gunnar S. Eriksen

In Norwegian cereals the concentrations of DON were extraordinary low and the concentrations of HT-2 and T-2 were extraordinary high in 2014. The result indicate that weather and agronomic means that suppress conditions for DON contamination may be of advantage for other mycotoxin producers such as* F. langsethiae *producing HT-2 and T-2. The concentrations of selected mycotoxins (DON, HT-2, T-2, nivalenol, zearalenone, ochratoxin A) in feed for pigs and poultry were below maximum recommended levels in all samples.

Introduction

The most important mycotoxin-producing moulds infecting cereals in the field during the growing season belong to the genus *Fusarium*. The most relevant mycotoxins produced by these moulds are the trichothecenes deoxynivalenol (DON), HT-2 toxin, T-2 toxin and nivalenol, as well as zearalenone and fumonisins. DON has been surveyed in Norwegian cereals for about two decades and has been found to occur in Norwegian cereals at high concentrations, particularly in oats and wheat. DON is considered as a health risk if affected cereal is ingested by animals and humans [1]. The prominent effects of DON exposure are gastrointestinal related with reductions of feed intake and growth rate, most pronounced in pigs. Also HT-2 and T-2 may be present at considerable concentrations in oats and products containing oats. HT-2 and T-2 have similar but stronger toxic effect potential than DON, and can cause gastrointestinal lesions as well as immune suppression [1]. Nivalenol, however, has usually been present at concentrations below the level of health risk. The oestrogenic mycotoxin zearalenone is produced by the same *Fusarium* species as DON but at concentrations at lower significance in Norway so far [1]. Fumonisins are produced by *Fusarium* species that infect maize. Although maize production is low in Norway and fumonisin producing Fusaria prefer more tropical climate [1], fumonisins in imported maize can constitute a health risk.

The most important mycotoxin-producing moulds that infect cereals and feed during storage are species of genera *Penicillium* and *Aspergillus*. *Penicillium* species grow and produce mycotoxins at lower temperatures than species of *Aspergillus*. Thus, Penicillia are the main genus of concern under our storage conditions while Aspergilli mainly occur in tropical areas. Ochratoxin A is an important mycotoxin produced by several species of both *Penicillium* and *Aspergillus* worldwide. The most prominent livestock effect of ochratoxin A is nephrotoxicity in pigs. The toxin may also suppress the immune function and performance [1]. So far, ochratoxin A has not caused problems for Norwegian husbandry. Nevertheless, active surveillance of ochratoxin is important because its potential risk and occurrence in imported feed ingredients is unpredictable [1]. Aflatoxins are produced by some Aspergilli and are considered a possible import problem for Norway. The carcinogenic and liver toxic aflatoxins in feed, particular in feed for dairy cattle, must be kept below the health risk level because an active aflatoxin metabolite can be found in milk for distributed for human consumption.

Aims

The aims of the programme are to provide reliable documentation of the occurrence of mould and selected important mycotoxins in cereals for feed and in complete feed in Norway, and to use this as a tool to reduce risk connected to animal mycotoxicoses and human mycotoxin exposure via animal products.

Materials and methods

The surveillance programme for mould and mycotoxins in feed materials and processed complete feed is collaboration between the Norwegian Food Safety Authority (NFSA) and the Norwegian Veterinary Institute (NVI) where NFSA decides the extent of the surveillance programme based on scientific advices from NVI. NFSA collects the samples and NVI is responsible for analysing and reporting of the results.

In 2014 the surveillance programme for feed consisted of the following samples received at NVI: a) 120 samples of small cereal grain consisting of oats, wheat and barley, b) 10 samples of maize and c) 44 samples of complete feed (for pigs and poultry). The number of samples of small grains followed the original plan for the programme. The sample number of maize was lower than planned (30 samples). The number of feed samples was somewhat higher than planned (34 samples). The samples were collected by the NFSA according to a specific plan for sampling where the various regional offices were involved. Samples of small grains were collected at mills in grain production areas and received at NVI during August to November. Samples of maize were collected from batches imported from 3.countries (outside EU) and were received at NVI during June to December. The samples of feed for pigs and poultry were collected with the feed industry organisations and received NVI throughout the year.

All samples of cereal grain (oats, wheat, barley) were analysed for the trichothecenes DON, HT-2, T-2 and nivalenol. In addition 30 of the oat samples were analysed for mould and *Fusarium*. The maize samples were analysed for aflatoxins and fumonisins. The samples of feed for pigs and poultry were analysed for the trichothecenes, zearalenone and ochratoxin A.

Quantitative determination of mould and *Fusarium*

Quantitative determinations of mould and *Fusarium* in 30 samples of oats were done according to NMKL method No 98, where only Malt-yeast-extract-sucrose-agar (MYSA) was used as growth medium. In addition to the total amount of mould it was done a qualitative determination of the composition of the mycoflora. *Fusarium* and storage fungi were counted separately. The detection limit for mould in feed was 50 cfu/g.

Identification of *Fusarium* species

The different *Fusarium* species found in oat were transferred to Potato Sucrose Agar (PSA) and Spezieller Nährstoffarmer Agar (SNA) with filter paper, for further identification. PSA plates were incubated in darkness at 25 °C in four to seven days. SNA with filter paper were incubated in seven days in combined black light and cold day light alternating with darkness, using 12 hours photoperiods. The *Fusarium* was identified to species level on basis of morphological characteristics. Some of the species were verified by using molecular method (PCR and DNA-sequencing).

Chemical analysis

Some of the samples were analysed at NVIs laboratory in Oslo, but due to technical and staff problems most samples were sent to Premier Analytical Services (PAS) in UK for analyses. Both laboratories are accredited for mycotoxin analyses in the relevant matrices and use similar methods.

The trichothecene mycotoxins deoxynivalenol, nivalenol, T-2 toxin and HT-2 toxin were determined by gas chromatography with mass spectrometry (GC/MS). The limit of quantification was 10 µg/kg for all these trichothecenes.

Zearalenone was analysed using immunoaffinity columns for clean-up followed by determination by HPLC with fluorescence detection. The limit of quantification was 3.0 µg/kg.

Ochratoxin A was analysed using immunoaffinity clean-up followed by determination by HPLC with fluorescence detection. The limit of quantification for ochratoxin was 0.01 µg/kg.

Fumonisin B1 and B2 were determined by LC-MS/MS using a UKAS accredited method with a limit of quantification of 10 µg/kg for each fumonisin.

Aflatoxins (B1, B2, G1, G2) have been analysed using immunoaffinity columns clean up followed by determination by HPLC using fluorescence detection after post-column derivatisation. The quantification limit for aflatoxins was 0.1 µg/kg.

Statistics

One-way analysis of variance (ANOVA) was used to compare the means of concentrations of toxins/grain type between geographical regions using the F-distribution. If the null hypothesis of no difference between the regions was rejected, pair-wise comparisons using the Sidak test were performed to identify statistical difference ($p<0.05$) between the regions. Pearson product-moment correlation coefficient (r), was used to measure the strength and direction of the linear relationships between two variables. The value of r lie between +1 and -1, where 1 is total positive correlation, 0 is no correlation, and -1 is total negative correlation. For values below LOQ, half LOQ was used in the data treatment.

Statistical procedures were executed with software STATA version 12.0 (StataCorp LP, College Station, TX, USA).

Results

Cereals

The concentration levels of DON were extraordinary low in all small cereal grains tested in the 2014 season, and the levels of nivalenol remained low as in previous years. The concentration levels of HT-2 and T-2 were relatively higher in 2014, mainly in oats. The concentrations of the trichothecenes DON, HT-2, T-2 and nivalenol in oats were 3-30 times higher than in wheat and barley (Table 1). The mean concentration of DON in oats, wheat and barley were 261, 76 and 79 µg/kg, respectively. The mean sum of HT-2 and T-2 in oats, wheat and barley were 303, 14 and 24 µg/kg, respectively. The mean concentrations of nivalenol in oats, wheat and barley were 84, 8 and 24 µg/kg, respectively.

Total mould and total *Fusarium* measured in oats showed mean concentrations of 1.94 mill cfu/g and 78 500 cfu/g, respectively. In particular total *Fusarium* showed large variation between samples. One of the 30 samples had a concentration of *Fusarium* below the detection limit. However, this sample had a very high occurrence of yeast (26 mill cfu/g). In the remaining 29 samples, total *Fusarium* ranged from 5000 to 400 000 cfu/g. With regard to storage fungi only one sample had an occurrence of substantial amount (100 000 cfu/g). Nine samples contained sparse occurrence of storage fungi and 20 samples did not contain detectable amounts.

Table 1. Concentrations of total mould and *Fusarium* in oats, and concentrations of deoxynivalenol (DON), HT-2 toxin, nivalenol (NIV), T-2 toxin and the sum of HT-2 and T-2 toxin in oats, wheat and barley sampled in 2014.

	Mould cfu/g	*Fusarium* cfu/g	DON µg/kg	HT2 µg/kg	NIV µg/kg	T2 µg/kg	HT2+T2 µg/kg
Oats							
Number	30	30	40	40	40	40	40
Mean	1 940 000	78 500	261	197	84	106	303
Median	1 250 000	50 000	158	151	76	67	224
Minimum-maximum	85 000-16 000 000	<50-400 000	13-1 140	<10-1 060	10-250	<10-392	<20-1332
St. deviation	2 830 386	96 043	272	180	65	98	258
Wheat							
Number	n.a.	n.a.	39	39	39	39	39
Mean			76	<10	<10	<10	<20
Median			42	<10	<10	<10	<20
Min-max			<10-629	<10-22	<10-47	<10-29	<20-51
St. deviation			113	5	8	5	8
Barley							
Number	n.a.	n.a.	41	41	41	41	41
Mean			79	17	24	<10	24
Median			32	16	12	<10	21
Min-max			<10-635	<10-48	<10-111	<10-20	<20-62
St. deviation			137	12	26	4	15

The testing for correlations between total *Fusarium* and trichothecenes in oats showed no correlation between total *Fusarium* content and DON (r=-0.09; N=30) and a weak positive correlation between total *Fusarium* and the sum of HT-2 and T-2 (r=0.36; N=30). These results reflect that the DON producing *Fusarium* species (*F. graminearum* and *F. culmorum*) showed low occurrence in 2014 whereas the dominant HT-2 and T-2 producing species (*F. langsethiae*) was more prevalent (Table 2).

The distribution of *Fusarium* species had a strikingly high proportion of *F. poae* and *F. langsethiae* and a similar low occurrence of *F. avenaceum* and *F. graminearum*. This differs remarkably from the occurrence of *Fusarium* species in recent years where *F. avenaceum* and *F. graminearum* have been the predominant species.

Table 2. The frequency of *Fusarium* species found in oats in 2014.

Species	Found in number (%) of samples, n=30	Number of samples with most dominant species*
F. poae	22 (73 %)	11
F. langsethiae	21 (70 %)	10
F. graminearum	8 (27 %)	0
F. tricinctum	5 (17 %)	3
F. avenaceum	5 (17 %)	1
F. culmorum	1 (3 %)	0

**F. poae* and/or *F. langsethiae* were the most dominant species in 24 samples (80 %). (Three samples with equal amount of both species.)

Determinations of correlations between mycotoxins showed a weak negative correlation (r=-0.19; N=40) between DON and the sum of HT-2 and T-2 in oats. Positive correlation between HT-2 and T-2 in oats was rather high (Figure 1).

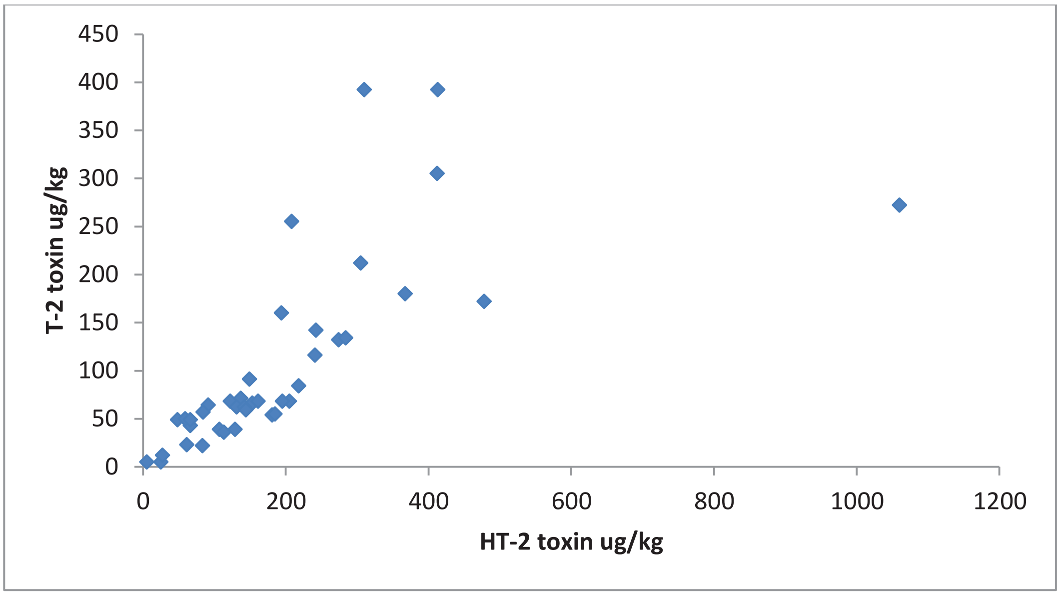

Figure 1. Correlation between HT-2 and T-2 toxin in oats sampled in 2014. Correlation coefficient r=0.70; N=40.

For oats from Trøndelag, the concentration levels of DON were higher and the levels of HT-2, T-2 and nivalenol lower than in the other regions (Table 3). For DON, one-way analysis of variance showed a statistically significant difference between regions. Pair-wise comparison between regions showed that only Trøndelag and Oslo/Akershus/Østfold had statistically significant difference (p=0.046). Neither HT-2+T-2 nor nivalenol in oats showed statistically significant regional differences. Wheat and barley did not show a similar tendency of geographic difference for trichothecenes as oats.

The maize samples were analysed for aflatoxins and fumonisins. The mean and maximum concentrations of aflatoxin B1 were low; 0.79 and 3.7 µg/kg. The mean and maximum concentrations of fumonisins (sum of fumonisins B1 and B2) were 2180 and 7410 µg/kg.

Complete feed

The concentrations of trichothecenes, zearalenone and ochratoxin A in feed for pigs and poultry are shown in Table 4. All mycotoxins showed lower mean concentrations in pig feed than in poultry feed. In feed for pigs, the mean concentrations of DON, sum HT-2+T-2, nivalenol, zearalenone and ochratoxin A were 208, 24, 18, 14, and 0.29 µg/kg, respectively. In feed for poultry, the mean concentrations of DON, sum HT-2+T-2, nivalenol, zearalenone and ochratoxin A were 309, 54, 46, 129, and 0.37 µg/kg, respectively.

Table 3. Regional survey of the concentrations of total mould and *Fusarium* in oats, and concentrations of deoxynivalenol (DON), HT-2 toxin, nivalenol (NIV), T-2 toxin and the sum of HT-2 and T-2 toxin (HT-2+T-2) in oats, wheat and barley sampled in 2014.

		*Fusarium** cfu/g	DON µg/kg	HT2 µg/kg	NIV µg/kg	T2 µg/kg	HT2+T2 µg/kg
Region Buskerud, Vestfold, Telemark							
Oats n=7/*n=5	Mean	99 000	193	293	101	122	415
	St.dev.	168 315	139	358	45	117	458
Wheat n=7	Mean		33	<10	14	<10	<20
	St.dev.		27	7	16	3	8
Barley n=10	Mean		51	19	46	<10	27
	St.dev.		35	13	36	5	18
Region Oslo, Akershus, Østfold							
Oats n=21/*n=20	Mean	86 501	189	188	92	119	307
	St.dev.	84 309	270	103	79	111	204
Wheat n=19	Mean		78	<10	<10	<10	<20
	St.dev.		146	2	6	4	5
Barley n=11	Mean		26	15	21	<10	20
	St.dev.		34	8	23	2	8
Region Hedmark, Oppland							
Oats n=7/*n=2	Mean	32 500	343	227	71	93	320
	St.dev.	24 749	240	131	20	55	183
Wheat n=8	Mean		115	<10	<10	<10	<20
	St.dev.		76	4	7	0	4
Barley n=10	Mean		199	27	19	10	36
	St.dev.		242	13	18	5	17
Region Trøndelag							
Oats n=5/*n=3	Mean	21 667	543	59	43	45	104
	St.dev.	24 664	305	34	48	23	57
Wheat n=5	Mean		63	11	<10	13	24
	St.dev.		98	8	0	10	17
Barley n=10	Mean		47	10	10	<10	<20
	St.dev.		38	6	9	0	6

Table 4. Concentrations of deoxynivalenol (DON), HT-2 toxin, nivalenol (NIV), T-2 toxin, the sum of HT-2 and T-2 toxin (HT-2+T-2), zearalenone (ZEA) and ochratoxin A (OTA) in complete feed for pigs and poultry sampled in 2014.

	DON µg/kg	HT-2 µg/kg	NIV µg/kg	T-2 µg/kg	HT2+T2 µg/kg	ZEA µg/kg	OTA µg/kg
Complete feed for pigs							
Number	24	24	24	24	24	24	24
Mean	208	15	18	<10	24	14	0.29
Median	191	10	15	<10	25	8	0.20
Min-max	68-496	<10-39	<10-64	<10-15	<20-53	<3-61	<0.1-2.6
St. deviation	104	11	12	5	13	19	0.50
Complete feed for poultry							
Number	20	20	20	20	20	20	20
Mean	309	38	46	15	54	129	0.37
Median	310	27	32	<10	33	125	0.23
Min-max	103-623	<10-229	11-224	<10-119	<20-348	9-233	<0.1-1.7
St. deviation	133	48	46	25	73	74	0.40

Discussion

Cereals

The concentration of DON in oats in 2014 was the lowest measured concentrations during the last decade (Figure 2). For wheat and barley, a comparison with previous years is more difficult as these cereal species have not been included consistently in the yearly surveillance programme. However, we can conclude that 2014 stands out as a year with extra low DON levels in the Norwegian cereal grains. The low DON contamination may be due to the warmer and dryer flowering season, July, of 2014 in the whole Norway compared to previous years [2]. The significantly higher DON concentrations in Trøndelag than in South-Eastern parts of Norway may be due to somewhat different local climate conditions in having lower temperatures or more precipitation than further south during the growth season.

The biggest challenge with respect to *Fusarium* head blight in Norwegian cereal production is oats. Although DON contamination was low in 2014, oat is also the preferred host of *F. langsethiae* [3, 4] and the concentrations of HT-2 and T-2 in oats were extraordinary high in 2014. The mean concentration of sum HT-2 and T-2 in oats has not shown such high levels since 2005 (Figure 3). However, these toxins were detected at low levels in wheat and barley, corresponding to the levels shown previous years [1]. HT-2 and T-2 are in Scandinavia first of all known to contaminate oats [4-6].

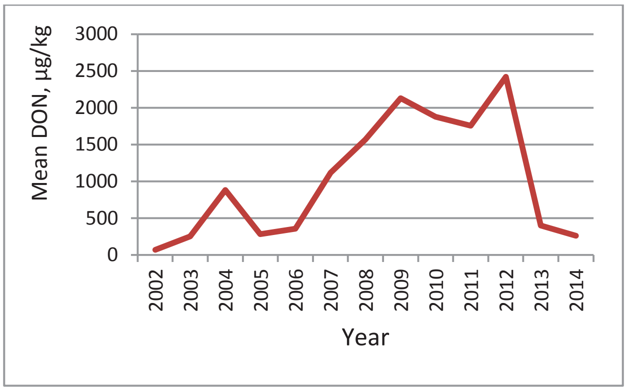

Figure 2. Mean concentration of deoxynivalenol (DON) in 30-60 samples of oats per year in the Norwegian surveillance programme for feed.

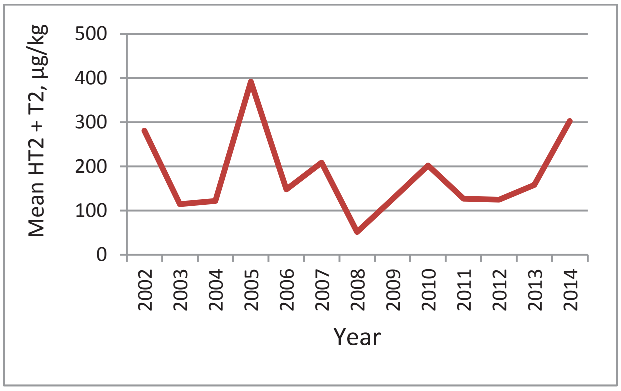

Figure 3. Mean concentration of the sum of HT-2 toxin and T-2 toxin in 30-60 samples of oats per year in the Norwegian surveillance programme for feed.

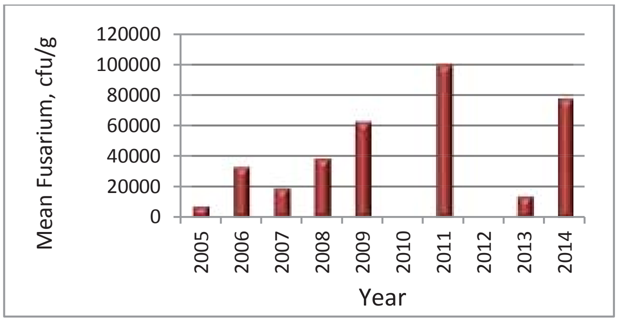

Figure 4. Mean concentration of total *Fusarium* in 25-40 samples of oats per year in the Norwegian surveillance programme for feed. In 2010 and 2012 quantitative determination of *Fusarium* was not included.

Looking at fungal data available for oat samples in 2014, we see a clear lack of correlation between the amount of DON and the total *Fusarium* content present in the samples. This indicates that the oat samples inconsistently contain *Fusarium* species which produce DON. On the other hand there is a positive correlation between HT-2+T-2 and *Fusarium*, indicating that the oat samples may be enriched with HT-2/T-2 producers such *as F. langsethiae*. For this reason, fungal analyses in oats for 2014 were extended beyond the requirement from the Norwegian Food Safety Authority, such that *Fusarium* was determined semi-quantitatively to the species level based on cultured samples. Our findings are interesting as they clearly indicate that indeed, *F. langsethiae* was one of the most abundant *Fusarium* species in the oat samples from 2014. That the possible relations between total *Fusarium* and DON or sum of HT-2+T-2 in oats varies year by year are illustrated in comparison of Figure 4 (total *Fusarium*) with Figure 2 (DON) and Figure 3 (HT-2+T-2).

Several scientists have opined that there seems to be a mutual exclusion between *F. graminearum* and *F. langsethiae*, and that *F. langsethiae* is more predominant during dryer summers [7, 8]. The mechanisms behind this are not fully understood, however, some data indicate that favourable conditions for *F. graminearum* will repress *F. langsethiae*, whereas unfavourable conditions and absence of *F. graminearum*, will allow for *F. langsethiae* to proliferate [9].

Based on NVI´s surveillance for NFSA, we have found several years where the HT-2/T-2 and DON levels in Norwegian oats seem to be inversely correlated (2005, 2008, 2012). A peak in HT-2/T-2 content similar to 2014 was observed in 2005. At this point however, the amount of *Fusarium* did not correlate with HT-2/T-2 and differentiated data on *Fusarium* species level do not exist. One reason for the absence of correlation may be a greater unawareness at the time for the *F. langsethiae* species and that it was overlooked during culturing due to overgrown colonies, and hence not counted in the total *Fusarium* number. Infestation of *F. langsethiae* positively influenced by temperature in July was observed in Norwegian cereals during 2002-2004 [10].

A trend indicating a kind of inverse relationship between HT-2+T-2 and DON contamination in Norwegian oats is supported by observations from scientists outside Norway [7]. However, there are also years where no significant inverse correlation between HT-2/T-2 and DON in Norwegian oats were shown [6]. The inverse correlation between DON and HT-2+T-2 in Norwegian oats in 2014 was rather weak, and indicates a 4 % (r^2) explanation of their respective variations. The implications of an inverse relationship should nevertheless be taken into consideration. Thus, in “good” years where DON contamination is low, there may be a reason to expect increased problems with HT-2/T-2 contamination. It also suggests that a one-sided focus on eradication of *F. graminearum*, may result in a proliferation and increased problems with *F. langsethiae*. Accentuating this scenario there is today no fungicide that has proven effective against *F. langsethiae* [11].

Fusarium infestations are largely dependent of the weather, and it is likely to expect that we will have similar oscillations between DON and HT-2/T-2 contaminations in Norwegian oats in the years to come.

In the samples of imported maize analysed for aflatoxins and fumonisins, all samples were well below the limits of maximum concentration of aflatoxin B1 at 20 µg/kg, and of the sum of fumonisin B1 and B2 at 60,000 µg/kg.

Complete feed

The mycotoxin concentrations showed an elevated level in feed for poultry than in feed for pigs in 2014. That is acceptable since poultry in general are less mycotoxin sensitive than pigs. These species differences are also reflected in the recommended maximum levels of the mycotoxins. None of the samples of feed for pigs showed concentrations of DON, sum HT-2+T-2, zearalenone and ochratoxin A above the recommended maximum levels. The maximum levels in feed for pigs are as follows: 500 µg/kg for DON, 200 µg/kg for HT-2 +T-2, 100 µg/kg for zearalenone in feed for piglets and young sows, and 250 µg/kg in feed for older sows and growing pigs, and 50 µg/kg for ochratoxin A. Trichothecenes determined in feed for pigs in 2013 [12] showed 1.6 times higher mean DON and 15 % of the samples above the recommended level, which are more similar with the DON-levels in pig feed shown in the previous years (2004-2011) [1]. The lower DON level in 2014 possibly reflects the low DON levels in cereals in 2013 and also that the feed industry had used improved tools to analyse DON in cereals and feed. The levels of the other trichothecenes were at a similar low level in pig feed in 2013 and 2014, which are some lower than shown in pig feed during previous years (2004-11) [1]. For zearalenone and ochratoxin A, comparisons with previous years are not possible due to lack of data on Norwegian pig feed.

Similarly, none of the samples of feed for poultry showed mycotoxin concentrations above these recommended maximum levels, except for one sample of feed for laying hens had exceeded the maximum level for sum HT-2+T-2 at 250 µg/kg. However, at the time for sampling the EU limit was probably not established in Norway. According to the Norwegian maximum level for sum HT-2+T-2 at 600 µg/kg, the sample containing 348 µg/kg did not exceed the limit. The maximum levels of the other mycotoxins in feed for poultry are as follows: 5000 µg/kg for DON, no limit for zearalenone, and 100 µg/kg for ochratoxin A. Mycotoxins were not determined in poultry feed in 2013 but some data collected from 2004-2011 [1] indicate a similar level of trichothecenes in 2014 as in previous years. For zearalenone and ochratoxin A, comparison with previous years was not possible due to lack of data on Norwegian poultry feed.

Conclusion

In a ten-year perspective, the concentrations of DON in Norwegian cereals were extraordinary low in 2014, whereas the concentrations of HT-2 and T-2 in oats were extraordinary high. The result may indicate that weather and agronomic means which suppress the conditions for DON contamination may be conductive for other mycotoxin producers such as *F. langsethiae* producing HT-2 and T-2.

The concentrations of selected mycotoxins (DON, HT-2, T-2, nivalenol, zearalenone, ochratoxin A) in feed for pigs and poultry showed concentrations below the maximum recommended levels in all samples.

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Appendix

Appendix Table 1. Concentrations of total mould and *Fusarium* in oats (30 samples), and concentrations of deoxynivalenol (DON), HT-2 toxin, nivalenol (NIV), T-2 toxin and the sum of HT-2 and T-2 toxin in oats (40 samples), wheat (39 samples) and barley (41 samples) in individual samples from different districts and regions in 2014.

District	ID	Cereal	Mould cfu/g	*Fusarium* cfu/g	DON µg/kg	HT-2 µg/kg	NIV µg/kg	T-2 µg/kg	HT-2+T-2, µg/kg
Region Buskerud, Vestfold, Telemark									
Hadeland og Ringerike	7259	Oats	1 200 000	30 000	72	412	162	305	717
Hadeland og Ringerike	7266	Oats	16 000 000	400 000	375	1 060	44	272	1 332
Hadeland og Ringerike	7270	Oats			163	149	119	91	240
Hadeland og Ringerike	7274	Oats			347	113	118	36	149
Vestfold	6543	Oats	1 100 000	25 000	96	153	42	66	219
Vestfold	6547	Oats	750 000	20 000	273	137	91	71	208
Vestfold	2263	Oats	590 000	20 000	24	27	134	12	39
Hadeland og Ringerike	7261	Wheat			43	22	19	<10	27
Hadeland og Ringerike	7267	Wheat			<10	<10	47	<10	<20
Hadeland og Ringerike	7272	Wheat			24	14	5	13	27
Vestfold	6544	Wheat			<10	<10	<10	<10	<20
Vestfold	6549	Wheat			38	<10	<10	<10	<20
Vestfold	6551	Wheat			83	<10	<10	<10	<20
Vestfold	6550	Wheat			35	<10	12	<10	<20
Hadeland og Ringerike	7258	Barley			19	13	11	<10	18
Hadeland og Ringerike	7268	Barley			77	<10	83	<10	<20
Hadeland og Ringerike	7271	Barley			119	48	73	14	62
Hadeland og Ringerike	7273	Barley			61	32	70	20	52
Hadeland og Ringerike	7275	Barley			55	21	17	12	33
Vestfold	6542	Barley			17	16	41	<10	21
Vestfold	6548	Barley			21	15	23	<10	20
Vestfold	6552	Barley			17	10	5	<10	<20
Vestfold	2264	Barley			86	<10	22	<10	<20
Vestfold	6527	Barley			40	25	111	<10	30
Region Oslo, Akershus, Østfold									
Romerike	6403	Oats	3 100 000	60 000	127	218	17	84	302
Romerike	3203	Oats	1 600 000	50 000	200	181	194	54	235
Romerike	3206	Oats	1 500 000	200 000	111	161	140	68	229
Romerike	6412	Oats	1 500 000	180 000	86	129	250	39	168
Romerike	6413	Oats	1 200 000	360 000	40	25	149	<10	30
Romerike	6416	Oats	2 600 000	140 000	786	107	115	39	146
Romerike	6417	Oats			252	205	77	68	273
Indre Østfold og Follo	2222	Oats	3 700 000	100 000	62	274	85	132	406
Indre Østfold og Follo	2246	Oats	150 000	5 000	1 140	66	14	43	109
Indre Østfold og Follo	2247	Oats	1 500 000	<50	48	83	152	22	105
Indre Østfold og Follo	4265	Oats	1 800 000	50 000	149	59	15	50	109
Indre Østfold og Follo	4267	Oats	1 300 000	50 000	82	367	10	180	547
Indre Østfold og Follo	4270	Oats	2 700 000	55 000	150	241	60	116	357
Ytre Østfold	2374	Oats	2 200 000	50 000	66	413	55	392	805
Ytre Østfold	2375	Oats	3 100 000	100 000	132	208	185	255	463
Ytre Østfold	2378	Oats	1 200 000	45 000	94	122	98	68	190
Ytre Østfold	6268	Oats	1 000 000	10 000	100	305	23	212	517
Ytre Østfold	6269	Oats	2 400 000	100 000	27	194	12	160	354
Ytre Østfold	5482	Oats	2 800 000	100 000	126	310	245	392	702
Ytre Østfold	7321	Oats	140 000	10 000	13	131	17	62	193
Ytre Østfold	7324	Oats	1 200 000	65 000	188	142	21	62	204
Romerike	3202	Wheat			21	12	<10	<10	<20

Romerike	6410	Wheat			114	<10	<10	<10	<20
Romerike	6411	Wheat			28	<10	<10	<10	<20
Romerike	6414	Wheat			629	<10	<10	<10	<20
Romerike	6415	Wheat			52	<10	<10	<10	<20
Romerike	6408	Wheat			<10	<10	<10	16	21
Romerike	6409	Wheat			24	<10	<10	16	21
Indre Østfold og Follo	2239	Wheat			186	<10	<10	<10	<20
Indre Østfold og Follo	2245	Wheat			<10	<10	<10	<10	<20
Indre Østfold og Follo	2248	Wheat			<10	<10	11	<10	<20
Indre Østfold og Follo	4269	Wheat			44	<10	<10	<10	<20
Indre Østfold og Follo	4271	Wheat			47	<10	<10	<10	<20
Indre Østfold og Follo	4282	Wheat			50	<10	<10	<10	<20
Ytre Østfold	2376	Wheat			55	<10	28	<10	<20
Ytre Østfold	2379	Wheat			182	<10	10	<10	<20
Ytre Østfold	6270	Wheat			<10	13	5	14	27
Ytre Østfold	5481	Wheat			<10	<10	15	<10	<20
Ytre Østfold	5464	Wheat			<10	<10	11	<10	<20
Ytre Østfold	7322	Wheat			16	<10	<10	<10	<20
Romerike	3204	Barley			15	21	<10	<10	26
Romerike	3205	Barley			14	14	59	<10	<20
Romerike	3207	Barley			23	<10	11	<10	<20
Indre Østfold og Follo	2223	Barley			<10	19	11	<10	24
Indre Østfold og Follo	2249	Barley			15	18	21	<10	23
Indre Østfold og Follo	4266	Barley			13	16	<10	10	26
Indre Østfold og Follo	4268	Barley			22	<10	<10	<10	<20
Indre Østfold og Follo	4283	Barley			126	15	<10	<10	20
Ytre Østfold	2377	Barley			16	12	67	<10	<20
Ytre Østfold	6267	Barley			29	<10	10	<10	<20
Ytre Østfold	5466	Barley			11	32	34	<10	37
Region Hedmark, Oppland									
Gudbrandsdal	6607	Oats			221	185	88	55	240
Gudbrandsdal	6608	Oats			251	478	78	172	650
Gudbrandsdal	6611	Oats			168	242	58	142	384
Øst-Hedmark	5490	Oats			639	144	74	59	203
Øst-Hedmark	5491	Oats			738	195	102	68	263
Valdres og Gjøvikregionen	5531	Oats	410 000	15 000	158	61	43	23	84
Valdres og Gjøvikregionen	5532	Oats	860 000	50 000	227	284	55	134	418
Hedmarken	6620	Wheat			230	<10	<10	<10	<20
Hedmarken	6621	Wheat			55	<10	<10	<10	<20
Hedmarken	6622	Wheat			45	<10	<10	<10	<20
Hedmarken	6629	Wheat			207	<10	24	<10	<20
Hedmarken	6630	Wheat			151	<10	<10	<10	<20
Hedmarken	6631	Wheat			132	<10	<10	<10	<20
Valdres og Gjøvikregionen	5533	Wheat			59	17	<10	<10	22
Valdres og Gjøvikregionen	5534	Wheat			42	<10	<10	<10	<20
Gudbrandsdal	6605	Barley			43	18	32	<10	23
Gudbrandsdal	6606	Barley			143	12	<10	13	25
Gudbrandsdal	6609	Barley			579	34	<10	11	45
Gudbrandsdal	6610	Barley			66	10	<10	<10	<20
Øst-Hedmark	5489	Barley			390	42	15	12	54
Øst-Hedmark	5492	Barley			635	42	32	17	59
Hedmarken	6618	Barley			32	31	<10	<10	36
Hedmarken	6619	Barley			36	14	17	<10	<20

Valdres og Gjøvikregionen	5535	Barley			22	20	12	<10	25
Valdres og Gjøvikregionen	5536	Barley			43	42	60	18	60
Region Trøndelag									
Sør-Innherred	7063	Oats	310 000	5 000	561	91	18	64	155
Sør-Innherred	7064	Oats	320 000	50 000	1010	48	15	49	97
Sør-Innherred	7065	Oats	85 000	10 000	469	84	129	57	141
Sør-Innherred	7071	Oats			519	66	26	49	115
Sør-Innherred	7086	Oats			158	<10	28	<10	<20
Sør-Innherred	7093	Wheat			238	<10	<10	<10	<20
Sør-Innherred	7094	Wheat			31	<10	<10	<10	<20
Sør-Innherred	7100	Wheat			26	16	<10	11	27
Sør-Innherred	7101	Wheat			16	<10	<10	15	20
Sør-Innherred	7102	Wheat			5	22	<10	29	51
Sør-Innherred	6849	Barley			12	17	<10	<10	22
Sør-Innherred	6850	Barley			46	<10	33	<10	<20
Sør-Innherred	6851	Barley			28	<10	<10	<10	<20
Sør-Innherred	7060	Barley			19	<10	<10	<10	<20
Sør-Innherred	7061	Barley			5	<10	12	<10	<20
Sør-Innherred	7062	Barley			78	16	<10	<10	21
Sør-Innherred	7072	Barley			61	<10	<10	<10	<20
Sør-Innherred	7073	Barley			117	20	11	<10	25
Sør-Innherred	7091	Barley			88	16	12	<10	21
Sør-Innherred	7116	Barley			11	<10	<10	<10	<20

Appendix Table 2. Concentrations of deoxynivalenol (DON), HT-2 toxin, nivalenol (NIV), T-2 toxin, the sum of HT-2 and T-2 toxin, zearalenone (ZEA) and ochratoxin A (OTA) in individual samples of complete feed for pigs (24 samples) and poultry (20 samples) in 2014.

Complete feed for pigs									
District	**ID**	**Type of feed**	**DON µg/kg**	**HT-2 µg/kg**	**NIV µg/kg**	**T-2 µg/kg**	**HT2+T2 µg/kg**	**ZEA µg/kg**	**OTA µg/kg**
Midt-Rogaland	3799	Growing pig	237	24	22	<10	29	5.7	0.20
Midt-Rogaland	3804	Piglets	108	14	17	<10	19	60.7	0.40
Midt-Rogaland	6574	Piglets	132	10	25	<10	15	<3.0	0.32
Midt-Rogaland	6575	Sow	239	14	36	<10	19	<3.0	0.28
Midt-Rogaland	6576	Growing pig	234	<10	21	<10	<20	9.4	0.29
Midt-Rogaland	6675	-	68	<10	<10	<10	<20	12.5	0.10
Haugalandet	9888	Sow	163	<10	13	<10	<20	4.4	0.27
Hardanger og Voss	6926	Growing pig	176	22	64	<10	27	4.9	0.19
Vestfold	6555	Sow	394	10	15	15	25	38.6	0.10
Indre Østfold og Follo	2218	Growing pig	146	26	23	<10	31	6.2	0.34
Ytre Østfold	5458	Growing pig	150	39	17	11	50	<3.0	<0.10
Ytre Østfold	7320	Sow	92	20	19	<10	25	4.6	0.10
Gudbrandsdal	0512	Growing pig	285	10	15	15	25	8.0	0.20
Hedmarken	2958	Growing pig	70	<10	<10	<10	<20	<3.0	<0.10
Sør-Innherred	1314	Growing pig	496	10	15	15	25	9.7	0.20
Sør-Innherred	3343	Growing pig	168	27	<10	10	37	5.1	2.60
Sør-Innherred	3538	Piglets	256	10	15	15	25	78.8	0.20
Sør-Innherred	3540	Sow	241	10	15	15	25	14.1	0.10
Sør-Innherred	3553	Growing pig	206	10	15	15	25	11.4	0.20
Sør-Innherred	3588	Sow	233	13	12	<10	18	3.9	0.20
Sør-Innherred	6826	Piglets	176	<10	11	<10	<20	24.2	0.10
Sør-Innherred	6830	Piglets	87	<10	<10	<10	<20	14.6	<0.10
Sør-Innherred	6838	Growing pig	299	32	14	15	47	9.3	0.30
Sør-Innherred	6841	Growing pig	332	39	24	14	53	8.3	0.20

Complete feed for poultry									
District	**ID**	**Type of feed**	**DON µg/kg**	**HT-2 µg/kg**	**NIV µg/kg**	**T-2 µg/kg**	**HT2+T2 µg/kg**	**ZEA µg/kg**	**OTA µg/kg**
Midt-Rogaland	3806	Broiler	440	51	27	20	71	130.5	1.70
Midt-Rogaland	3807	Broiler	225	27	25	<10	32	33.7	0.80
Midt-Rogaland	3808	Laying hen	623	51	57	<10	56	54.7	0.30
Midt-Rogaland	6671	Laying hen	416	16	24	<10	21	117.3	0.10
Midt-Rogaland	6672	Broiler	186	<10	11	<10	<20	58	0.10
Vestfold	2269	Broiler	290	17	45	13	30	232.8	0.70
Vestfold	6565	Broiler	222	65	28	32	97	8.8	0.10
Indre Østfold og Follo	2219	Laying hen	451	28	40	<10	33	308	0.55
Indre Østfold og Follo	4273	Broiler	445	17	69	12	29	119.7	0.10
Indre Østfold og Follo	4276	Turkey	360	20	84	<10	25	95.2	0.10
Ytre Østfold	5453	Broiler	203	23	39	<10	28	138.2	0.13
Ytre Østfold	5454	Broiler	110	16	224	<10	21	142	0.10
Ytre Østfold	5459	Laying hen	238	26	22	11	37	113.7	0.13
Ytre Østfold	5460	Broiler	180	<10	15	<10	<20	178.6	0.29
Ytre Østfold	5484	Broiler	365	30	26	13	43	145	0.20
Valdres og Gjøvik	5528	Laying hen	103	229	22	119	348	71.2	0.80
Sør-Innherred	1296	Laying hen	404	27	58	10	37	136.1	0.26
Sør-Innherred	6828	Turkey	236	51	30	17	68	61	<0.10
Sør-Innherred	7089	Laying hen	330	45	40	10	55	210.1	0.50
Sør-Innherred	7096	Broiler	357	17	34	<10	22	222.5	0.40

Appendix Table 3. Concentrations of aflatoxin B1, B2, G1, G2, fumonisin B1, B2 and the sum of fumonisin B1 and B2 in maize (10 samples) in 2014. All concentrations in µg/kg.

District	ID	Raw material	Afla B1	Afla B2	Afla G1	Afla G2	Sum Afla	Fum B1	Fum B2	FumB1+FumB2
Midt-Rogaland	6676	Maize	<0.10	<0.10	<0.10	<0.10	<0.40	18	< 10	23
Vestfold	2265	Maize	0.10	<0.10	<0.10	<0.10	<0.40	180	22	202
Ytre Østfold	5461	Maize	<0.10	<0.10	<0.10	<0.10	<0.40	553	124	677
Ytre Østfold	5483	Maize	0.10	<0.10	<0.10	<0.10	<0.40	184	34	218
Ytre Østfold	7323	Maize	<0.10	<0.10	<0.10	<0.10	<0.40	1 860	433	2 293
Ytre Østfold	6271	Maize	<0.10	<0.10	<0.10	<0.10	<0.40	241	46	287
Ytre Østfold	6272	Maize	0.10	<0.10	<0.10	<0.10	<0.40	1 970	437	2 407
Sør-Innherred	6848	Maize	0.30	<0.10	<0.10	<0.10	0.45	827	139	966
Sør-Innherred	7392	Maize	3.40	0.30	0.10	<0.10	3.85	3 990	3 420	7 410
Sør-Innherred	7095	Maize	3.70	0.30	0.10	<0.10	4.15	4 070	3 221	7 291
Mean			0.79	0.10	<0.10	<0.10	1.00	1 389	788	2 177
Median			0.10	<0.10	<0.10	<0.10	<0.40	690	132	822
St. deviation			1.46	0.11	0.02	0.00	1.58	1 551	1 345	2 853

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