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The surveillance programme for mould and mycotoxins in feed materials and complete feed in Norway 2015

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The surveillance programme for mycotoxins and fungi in feed materials, and complete and complementary feed in Norway 2015

Aksel Bernhoft, Ellen Christensen, Morten Sandvik

In 2015 low concentrations of DON were shown in wheat, oats and barley, whereas high concentrations of HT-2 and T-2 were shown in oats. Aflatoxin above the permitted limit was found in samples of maize. Very satisfactory results were shown for mycotoxins in feed: Aflatoxins in feed for ruminants were not detected, and no samples of feed for pigs contained mycotoxins (DON, HT-2, T-2, nivalenol, zearalenone, ochratoxin) above the maximum recommended limits. However, high amounts of storage mould and yeast were shown in samples of farm-mixed feed, particularly for ruminants, indicating inacceptable quality.

Introduction

The most important mycotoxin-producing moulds primarily 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 contaminated cereal is ingested by animals and humans [1]. The prominent effects of DON exposure are gastrointestinal signs related with reductions of feed intake and growth rate, particularly well documented 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 usually shown at insignificant concentrations in Norwegian cereals [1]. However the occurrence data on zearalenone are sparse. Fumonisins are produced by *Fusarium* species infecting maize. Although maize production is low in Norway and fumonisin producing Fusaria prefer more continental climate [1], fumonisins in imported maize may constitute a health risk.

The most important mycotoxin-producing moulds that infect cereals and feed primarily during storage are species of genera *Penicillium* and *Aspergillus*. *Penicillium* species generally grow and produce mycotoxins at lower temperatures than species of *Aspergillus*. Thus, Penicillia are the main genus of concern under Norwegian 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 must be kept below levels implying human health risk via consumption of animal products. Of particular concern are aflatoxins in feed for dairy cattle because an active aflatoxin metabolite can be present in milk distributed for human consumption.

Aims

The aims of the programme are to provide reliable documentation of the occurrence of important mycotoxins and fungi in feed cereal materials, and complete and complementary feed in Norway, and to use the data to assess risk connected to animal mycotoxicoses and human mycotoxin exposure via animal products.

Materials and methods

The surveillance programme for mycotoxins and fungi in feed materials and complete feed is a 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 is responsible for collecting the samples and NVI for analysing and reporting of the results.

In 2015 the surveillance programme for feed consisted of the following samples received at NVI: a) 101 samples of small cereal grain consisting of oats (50), barley (26) and wheat (25), b) 13 samples of maize/maize gluten, c) 38 samples of complementary compound feed for ruminants, d) 30 samples of complete compound feed for pigs, and e) 29 samples of complete feed prepared at the farms consisting of mixed feed for ruminants (14) and mixed wet feed for pigs (15). The sample number of maize was lower than planned (30 samples). The number of the other samples followed in general the original plan for the programme: 100 samples of small cereal grain, and respectively 40, 30 and 30 samples of ruminant compound feed, pig compound feed, and farm-mixed feed.

The samples were collected by NFSA according to a specific plan for sampling where the various regions were involved. Samples of small grains were collected at mills in grain production areas and received at NVI during September to November. Samples of maize were collected from batches imported from 3.countries and were received at NVI during April to December. The samples of compound feed for ruminants and pigs were collected with the feed industry organisations and received NVI throughout the year. The samples of farm-mixed feed were collected at the farms and received NVI during October and November, except a single sample received in March. The sampling was done according to EU Regulation 691/2013, with the purpose of having representative samples. The sampling procedures take into account size/volume of the lot to be sampled, if the substances to be analyzed are likely to be uniformly or non-uniformly distributed, number of incremental samples, sampling tools, size of final samples etc.

All samples of cereal grain (oats, barley, wheat) were analysed for the trichothecenes DON, HT-2, T-2 and nivalenol. The maize samples were analysed for aflatoxins and fumonisins. The samples of compound feed for ruminants were analysed for aflatoxins, whereas the compound feed for pigs were analysed for trichothecenes, zearalenone and ochratoxin A. The samples of farm-mixed feed were analysed for mould, yeast and trichothecenes.

Quantitative determination of fungi

Quantitative determinations of mould and yeast in the samples of complete feed prepared at the farms were done according to NMKL method No 98, where only Dichloran-Glycerol (DG18) was used as growth medium. In addition to the total amount of mould it was done a qualitative determination of the composition of the mycobiota. Field mould, storage mould and yeast were counted separately. The detection limit for mould and yeast in feed was 50 cfu/g.

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 (UK).

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 (UK).

Ochratoxin A was analysed using immunoaffinity clean-up followed by determination by HPLC with fluorescence detection. The limit of quantification for ochratoxin was 0.1 µg/kg in UK, and 0.05 in Oslo.

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 (UK).

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 all aflatoxins was 0.1 µg/kg in UK and 0.3-0.75 in Oslo.

Results

Cereals

The concentration levels of DON were low in all small cereal grains tested in the 2015 season, and the levels of nivalenol remained low as in previous years. As usual, oats showed higher DON concentrations than wheat and barley and the mean concentrations in oats, wheat and barley were 174, 25 and 37 µg/kg, respectively (Table 1). The concentration levels of HT-2 and T-2 were relatively high in 2015, mainly in oats. The mean sum of HT-2 and T-2 in oats, wheat and barley were 255, <20 and 37 µg/kg, respectively. The concentrations of nivalenol were insignificant.

Table 1. Concentrations (µg/kg) of deoxynivalenol (DON), nivalenol (NIV), HT-2 toxin, T-2 toxin and the sum of HT-2 and T-2 toxin in oats, wheat and barley sampled in 2015.

	DON	NIV	HT-2	T-2	HT-2+T-2
Oats					
Number	50	50	50	50	50
Mean	**174**	**23**	**166**	**89**	**255**
Median	86	16	127	62	186
Min-max	<10-1,140	<10-74	<10-1,090	<10-698	<20-1,788
St. deviation	214	17	185	111	295
Wheat					
Number	25	25	25	25	25
Mean	**25**	**<10**	**<10**	**<10**	**<20**
Median	22	<10	<10	<10	<20
Min-max	<10-94	<10-12	<10-23	<10-12	<20-34
St. deviation	22	2	5	2	7
Barley					
Number	26	26	26	26	26
Mean	**37**	**11**	**24**	**13**	**37**
Median	29	<10	<10	<10	<20
Min-max	<10-137	<10-62	<10-142	<10-93	<20-235
St. deviation	34	12	34	21	52

Oats from region Midt (Trøndelag, Møre and Romsdal) showed lower concentrations of the sum of HT-2 and T-2 than oats from the regions in South-Eastern Norway (Table 2). There were no clear geographical differences for other mycotoxins in oats or for any mycotoxins in wheat and barley.

Table 2. Regional survey of the concentrations (µg/kg) of deoxynivalenol (DON), nivalenol (NIV), HT-2 toxin, T-2 toxin and the sum of HT-2 and T-2 toxin (HT-2+T-2) in oats, wheat and barley sampled in 2015.

		DON	NIV	HT-2	T-2	HT-2+T-2
Region Øst (Buskerud, Vestfold, Telemark, Hedmark, Oppland)						
Oats n=20	Mean	**221**	**19**	**148**	**78**	**226**
	St. deviation	273	12	102	56	156
Wheat n=11	Mean	**24**	**<10**	**11**	**<10**	**<20**
	St. deviation	19	0	7	3	9
Barley n=10	Mean	**35**	**<10**	**21**	**14**	**34**
	St. deviation	42	6	43	28	71
Region Stor-Oslo (Akershus, Oslo, Østfold)						
Oats n=20	Mean	**136**	**32**	**252**	**141**	**393**
	St. deviation	119	20	243	150	392
Wheat n=10	Mean	**31**	**<10**	**<10**	**<10**	**<20**
	St. deviation	26	2	3	5	3
Barley n=8	Mean	**52**	**10**	**32**	**15**	**47**
	St. deviation	29	7	35	23	47
Region Midt (Nord-Trøndelag, Sør-Trøndelag, Møre og Romsdal)						
Oats n=10	Mean	**155**	**<10**	**27**	**10**	**37**
	St. deviation	229	5	25	6	30
Wheat n=4	Mean	**10**	**<10**	**<10**	**<10**	**<20**
	St. deviation	9	4	5	4	8
Barley n=8	Mean	**24**	**15**	**20**	**10**	**31**
	St. deviation	23	21	21	8	28

The maize samples were analysed for aflatoxins and fumonisins (Table 3). The mean and maximum concentrations of aflatoxin B1 were relatively high, 15.3 and 100.4 µg/kg, respectively. The explanation for the high mean concentration is two samples with extraordinary high aflatoxin level (aflatoxin B1 at 97.4 and 100.4 µg/kg) which is about 5 times above the maximum limit. The mean and maximum concentrations of fumonisins (sum of fumonisins B1 and B2) were 1,355 and 12,290 µg/kg, and no samples were above the recommended limit at 60,000 µg/kg [2].

Table 3. Concentrations (µg/kg) of aflatoxins B1, B2, G1, G2, sum aflatoxin, fumonisin B1, B2 and sum fumonisins in maize sampled in 2015.

	Afla B1	Afla B2	Afla G1	Afla G2	Sum Afla	Fum B1	Fum B2	FumB1+FumB2
Maize								
Number	13	13	13	13	13	13	13	13
Mean	**15.3**	**1.94**	**<0.10**	**<0.10**	**17.4**	**1001**	**354**	**1,355**
Median	0.10	<0.10	<0.10	<0.10	<0.40	223	58	279
Min-max	<0.1-100.4	<0.1-17.4	<0.1-<0.2	<0.1-<0.15	<0.4-115.0	31-8,750	<10-3,540	36-12,290
St. deviation	37.1	5.06	0.03	0.02	41.8	2355	961	3,314

Complete and complementary feed

Complementary compound feed for ruminants were analysed for aflatoxins. None of the 38 samples contained quantifiable concentrations of aflatoxins.

The concentrations of trichothecenes, zearalenone and ochratoxin A in complete compound feed for pigs are shown in Table 4, and trichothecenes as well as yeast and mould in wet, farm-mixed feed for pigs are shown in Table 5. Most samples of the compound feed for pigs showed detectable mycotoxin concentrations, however at an insignificant level. The mean mycotoxin concentrations of DON, nivalenol, sum HT-2+T-2, zearalenone and ochratoxin A were 117, 20, 65, 37 and 0.26 µg/kg, respectively. In the wet, farm-mixed complete feed for pigs, hardly no trichothecenes were detected. Two samples had a content of storage fungi above 25,000 cfu/g – indicating reduced hygienic quality [2]. The storage fungi were dominated by *Penicillium*, but also *Aspergillus* and Mucorales were found.

Concentrations of trichothecenes as well as yeast and mould in farm-mixed complete feed for ruminants are shown in Table 6. Only traces of trichothecenes were detected in some of the samples. Nine of the 14 samples had a content of yeast above 1,000,000 cfu/g – indicating reduced hygienic quality, and four of these samples showed above 10,000,000 cfu/g which indicate an inacceptable quality [2]. Furthermore, eight samples contained storage fungi above10,000 cfu/g (reduced hygienic quality), and four of them above 50,000 cfu/g (inacceptable quality) [2]. The storage fungi were mostly of the genus *Penicillium*, but in a few samples *Aspergillus* and Mucorales were also found. In total only three samples of farm-mixed complete feed for ruminants were of acceptable hygienic quality.

Table 4. Concentrations (µg/kg) of deoxynivalenol (DON), nivalenol (NIV), HT-2 toxin, T-2 toxin, the sum of HT-2 and T-2 toxin (HT-2+T-2), zearalenone (ZEA) and ochratoxin A (OTA) in complete compound feed for pigs sampled in 2015.

	DON	NIV	HT-2	T-2	HT 2+T 2	ZEA	OTA
Complete feed for pigs							
Number	30	30	30	30	30	30	30
Mean	**117**	**20**	**47**	**19**	**65**	**37**	**0.26**
Median	107	12	43	16	66	14	0.19
Min-max	20-289	<10-116	10-94	<10-60	<20-140	<3-217	<0.1-1.4
St. deviation	68	24	24	14	36	55	0.28

Table 5. Concentrations of yeast, mould, deoxynivalenol (DON), nivalenol (NIV), HT-2 toxin, T-2 toxin and the sum of HT-2 and T-2 toxin (HT-2+T-2) in complete farm-mixed feed for pigs sampled in 2015.

	Yeast cfu/g	Total mould cfu/g	Storage mould cfu/g	DON µg/kg	NIV µg/kg	HT-2 µg/kg	T-2 µg/kg	HT-2+T-2 µg/kg
Farm-mixed complete feed for pigs								
Number	15	15	15	15	15	15	15	15
Mean	**57,422**	**9,307**	**7,322**	**<10**	**<10**	**<10**	**<10**	**<20**
Median	2,800	350	350	<10	<10	<10	<10	<20
Min-max	<50-730,000	<50-60,000	<50-60,000	<10-34	<10	<10	<10	<20
St. deviation	186,925	19,228	16,220	8	0	1	0	1

Table 6. Concentrations of yeast, mould, deoxynivalenol (DON), nivalenol (NIV), HT-2 toxin, T-2 toxin and the sum of HT-2 and T-2 toxin (HT-2+T-2) in complete farm-mixed feed for ruminants sampled in 2015.

	Yeast cfu/g	Total mould cfu/g	Storage mould cfu/g	DON µg/kg	NIV µg/kg	HT-2 µg/kg	T-2 µg/kg	HT-2+T-2 µg/kg
Farm-mixed complete feed for ruminants								
Number	14	14	14	14	14	14	14	14
Mean	**18,625,800**	**62,142**	**45,356**	**26**	**<10**	**15**	**<10**	**20**
Median	4,000,000	12,500	10,000	22	<10	12	<10	<20
Min-max	<50-74,000,000	<50-280,000	<50-280,000	<10-79	<10-21	<10-36	<10	<20-41
St. deviation	25,824,016	96,297	80,186	23	6	11	0	11

Discussion

Cereals

The concentrations of DON in oats in 2015 were at the same low level or even lower than in 2014, which were the lowest measured concentrations during the last decade (Figure 1). The mean concentration of DON in oats in 2015 was 174 µg/kg compared with 261 µg/kg in 2014 [2]. As usual, oats showed significantly higher DON concentrations than wheat and barley. The DON levels in the various cereal species follow each other year by year. However, for wheat and barley, we do not have data for comparison for all years as these cereal species have not been consistently included in the yearly surveillance programmes. The DON results in wheat and barley in 2015 were at a similar low level or even lower than in in 2014. Thus, we can conclude that 2015 stands out as a year with extra low DON levels in the Norwegian cereal grains.

From Figure 1 we can see that the DON concentration in oats has been low during all three last years. The July months of 2013 and 2014 were warm and dry which may explain the favourable situation concerning DON [4, 5]. In contrast, July of 2015 was more cold and humid [6], and more DON in the cereals could have been expected. However, the last summer was rather windy, which probably reduced the field humidity and thus the DON levels.

The DON concentrations in cereals from the various regions were rather even and reflected the relatively uniform climate conditions in the regions during the growth season [6].

In 2015 the concentrations of HT-2 and T-2 in oats were high, similarly as in 2014 [3] which was a year of extraordinarily high concentrations of these trichothecenes (Figure 2). Oats from Trøndelag showed low concentrations of HT-2 and T-2 as commonly observed [1, 3, 7]. These mycotoxins were only present at low levels in barley and hardly present in wheat, which is a common finding in Scandinavia [1].

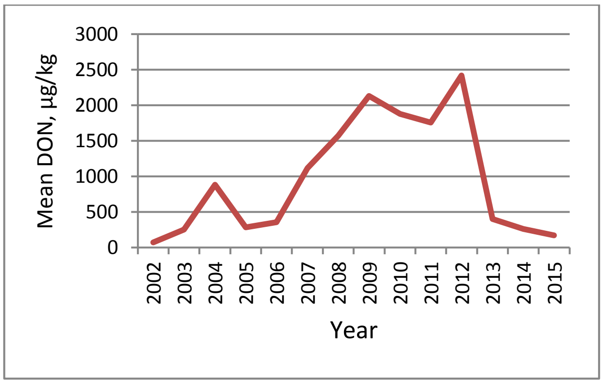

Figure 1. Mean concentration of deoxynivalenol (DON) in 30-60 samples of oats per year in the Norwegian surveillance programme for feed.

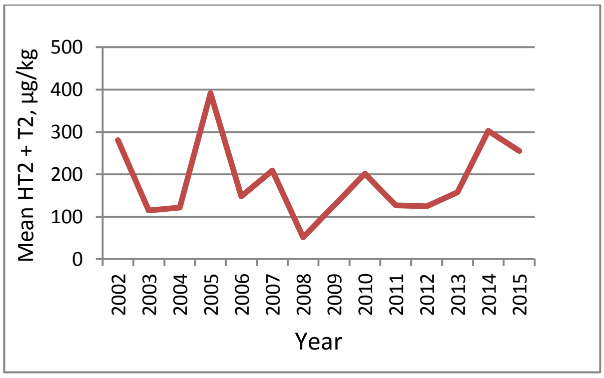

Figure 2. Mean concentration of the sum of HT-2 toxin and T-2 toxin in 30-60 samples of oats per year in the Norwegian surveillance programme for feed.

Fusarium graminearum is the main producer of DON and *F. langsethiae* the main producer of HT-2 and T-2 in Norwegian cereals [1]. 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 [8, 9]. 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 [10]. Figure 1 and 2 illustrate a kind of inverse mean concentrations of DON and HT-2 + T-2 in oats during 2002-15. 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].

In the samples of imported maize analysed for aflatoxins and fumonisins, two samples were about five times above the permitted limit of aflatoxin B1 at 20 µg/kg. The results illustrate the need to analyse feed batches of feed and ingredients imported from warmer zones. All the maize samples were below the recommended limit for the sum of fumonisin B1 and B2 at 60,000 µg/kg [2].

Complete and complementary feed

None of the samples of complementary compound feed for ruminants analyzed for aflatoxins contained quantifiable values. This is a satisfactory and also commonly shown result for these carcinogenic toxins [1]. The main purpose of the surveillance of aflatoxins in feed for ruminants is to avoid the risk for residues of aflatoxin metabolites in milk and dairy products for human consumption.

The complete compound feed for pigs showed low mycotoxin values with all results below the recommended maximum levels [2]. This is the same satisfactory situation as in 2014 [3]. However in 2013 and the decade before, the prevalence of samples of pig feed with concentrations of DON above the recommended levels was significant [1, 12]. The lower DON level in pig feed in 2014 and 2015 possibly reflects the low DON levels in cereals in 2013 and 2014 [3, 12] and also that the feed industry have established improved tools to analyse DON in cereals and feed. The levels of HT-2 and T-2 in the feed for pigs were somewhat higher in 2015 than in 2014 and the recent years before [1, 3, 12]. As for DON an explanation can be that the HT-2 and T-2 levels in the compound feed reflect the levels in cereal grain the year before: 2014 showed high levels of these toxins. For zearalenone in pig feed, the level in 2015 was about twice that in 2014 [3]. If the zearalenone level is mainly connected to Norwegian or imported feed ingredients is not known. For ochratoxin A the level in 2015 was at similar low and insignificant level as in 2014 [3]. For zearalenone and ochratoxin A comparisons with previous years are not possible due to lack of data on Norwegian pig feed.

The samples of farm-mixed complete feed for pigs and ruminants did not contain amounts of trichothecenes. Storage mould was, however, shown at considerable concentrations in several samples of feed for ruminants and in some samples of feed for pigs. The mould at certain levels may adversely affect animal health and several species of storage mould are also producers of mycotoxins or other bioactive compounds (13). In addition several of the farm-mixed feed for ruminants contained high concentrations of yeast which also reduce the hygienic quality of the feed. In total for the farm-mixed feed samples, only 3 out of 14 samples (21 %) of feed for ruminants and 13 out of 15 samples (87 %) were of acceptable quality.

Conclusions

- In 2015 the Norwegian cereals showed low concentrations of DON, whereas high concentrations of HT-2 and T-2 were shown in oats. These results are similar as in 2014. However, the summer of 2015 was cold and humid, whereas the summer of 2014 warm and dry. We suggest the more windy weather during the summer of 2015 may have dried the field and influenced the pattern *Fusarium* species and their mycotoxins produced.

- Aflatoxins above the permitted limit were found in 2 of 13 examined batches of maize as feed ingredient. The results illustrate the need for aflatoxin analyses on feed batches and ingredients imported from warmer zones.

- Very satisfactory results were shown for mycotoxins in feed produced by the Norwegian industry: Aflatoxins in feed for ruminants were not detected, and no feed for pigs contained mycotoxins (DON, HT-2, T-2, nivalenol, zearalenone, ochratoxin) above the maximum recommended levels.

- In farm-mixed feed for ruminants and pigs, considerable amounts of storage mould and yeast were shown. Only 21 % of the samples of feed for ruminants, and 87 % of the feed for pigs were of acceptable quality. Trichothecene mycotoxins were hardly detected in these samples.

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Appendix

Appendix Table 1. Concentrations of deoxynivalenol (DON), nivalenol (NIV), HT-2 toxin, T-2 toxin and the sum of HT-2 and T-2 toxin in oats (50 samples), wheat (25 samples) and barley (26 samples) in individual samples from different districts and regions in 2015. All concentrations in µg/kg.

Region	Cereal	DON	NIV	HT-2	T-2	HT-2
Region Midt	Oats	12	<10	20	11	31
Region Midt	Oats	<10	14	<10	<10	<20
Region Midt	Oats	83	16	48	10	58
Region Midt	Oats	661	14	18	<10	23
Region Midt	Oats	14	<10	13	<10	<20
Region Midt	Oats	90	13	85	25	110
Region Midt	Oats	20	<10	39	12	51
Region Midt	Oats	494	<10	10	10	20
Region Midt	Oats	119	<10	<10	<10	<20
Region Midt	Oats	54	10	23	13	36
Region Midt	Wheat	<10	<10	<10	<10	10
Region Midt	Wheat	<10	<10	<10	<10	10
Region Midt	Wheat	<10	12	13	11	24
Region Midt	Wheat	23	<10	13	12	25
Region Midt	Barley	52	<10	22	13	35
Region Midt	Barley	<10	<10	13	<10	<20
Region Midt	Barley	56	<10	<10	<10	<20
Region Midt	Barley	<10	30	67	19	86
Region Midt	Barley	19	<10	<10	<10	<20
Region Midt	Barley	<10	<10	<10	<10	<20
Region Midt	Barley	42	62	33	25	58
Region Midt	Barley	<10	<10	13	<10	<20
Region Stor-Oslo	Oats	330	24	1 090	698	1 788
Region Stor-Oslo	Oats	21	13	256	131	387
Region Stor-Oslo	Oats	71	39	210	102	312
Region Stor-Oslo	Oats	177	11	126	51	177
Region Stor-Oslo	Oats	280	63	640	324	964
Region Stor-Oslo	Oats	416	24	118	62	180
Region Stor-Oslo	Oats	143	74	158	76	234
Region Stor-Oslo	Oats	84	17	86	56	142
Region Stor-Oslo	Oats	41	<10	13	15	28
Region Stor-Oslo	Oats	29	55	283	173	456
Region Stor-Oslo	Oats	191	15	128	87	215
Region Stor-Oslo	Oats	71	29	195	64	259
Region Stor-Oslo	Oats	54	37	271	102	373
Region Stor-Oslo	Oats	234	72	293	171	464
Region Stor-Oslo	Oats	11	12	129	70	199
Region Stor-Oslo	Oats	213	29	90	73	163
Region Stor-Oslo	Oats	19	33	302	203	505
Region Stor-Oslo	Oats	244	41	442	209	651
Region Stor-Oslo	Oats	13	21	86	49	135
Region Stor-Oslo	Oats	71	33	129	98	227
Region Stor-Oslo	Wheat	37	<10	<10	<10	<20
Region Stor-Oslo	Wheat	<10	<10	<10	<10	<20
Region Stor-Oslo	Wheat	19	<10	10	<10	<20
Region Stor-Oslo	Wheat	22	10	<10	<10	<20
Region Stor-Oslo	Wheat	94	<10	<10	<10	<20
Region Stor-Oslo	Wheat	44	<10	12	<10	<20

Region Stor-Oslo	Wheat	22	<10	<10	<10	<20
Region Stor-Oslo	Wheat	13	<10	<10	<10	<20
Region Stor-Oslo	Wheat	12	<10	<10	<10	<20
Region Stor-Oslo	Wheat	41	<10	<10	<10	<20
Region Stor-Oslo	Barley	17	<10	20	<10	25
Region Stor-Oslo	Barley	22	18	17	<10	22
Region Stor-Oslo	Barley	77	14	21	<10	26
Region Stor-Oslo	Barley	50	<10	36	69	105
Region Stor-Oslo	Barley	49	<10	<10	<10	<20
Region Stor-Oslo	Barley	36	<10	<10	<10	<20
Region Stor-Oslo	Barley	104	<10	37	<10	42
Region Stor-Oslo	Barley	62	22	113	24	137
Region Øst	Oats	1 140	12	114	44	158
Region Øst	Oats	713	11	86	34	120
Region Øst	Oats	166	23	406	250	656
Region Øst	Oats	369	11	35	21	56
Region Øst	Oats	78	13	31	20	51
Region Øst	Oats	79	40	127	58	185
Region Øst	Oats	414	26	328	113	441
Region Øst	Oats	32	<10	49	20	69
Region Øst	Oats	163	12	234	132	366
Region Øst	Oats	44	<10	309	158	467
Region Øst	Oats	87	27	155	93	248
Region Øst	Oats	40	51	68	40	108
Region Øst	Oats	38	32	133	62	195
Region Øst	Oats	44	33	191	96	287
Region Øst	Oats	67	19	165	83	248
Region Øst	Oats	210	16	127	59	186
Region Øst	Oats	88	19	165	106	271
Region Øst	Oats	211	15	70	44	114
Region Øst	Oats	221	12	113	82	195
Region Øst	Oats	222	<10	59	38	97
Region Øst	Wheat	40	<10	<10	<10	<20
Region Øst	Wheat	33	<10	15	<10	20
Region Øst	Wheat	64	<10	15	<10	20
Region Øst	Wheat	38	<10	23	11	34
Region Øst	Wheat	15	<10	20	12	32
Region Øst	Wheat	35	<10	14	<10	<20
Region Øst	Wheat	5	<10	<10	<10	<20
Region Øst	Wheat	5	<10	<10	<10	<20
Region Øst	Wheat	5	<10	<10	<10	<20
Region Øst	Wheat	23	<10	<10	<10	<20
Region Øst	Wheat	5	<10	<10	<10	<20
Region Øst	Barley	137	<10	<10	<10	<20
Region Øst	Barley	<10	<10	<10	<10	<20
Region Øst	Barley	34	<10	<10	<10	<20
Region Øst	Barley	10	11	24	<10	29
Region Øst	Barley	64	20	142	93	235
Region Øst	Barley	<10	<10	<10	<10	<20
Region Øst	Barley	23	<10	<10	<10	<20
Region Øst	Barley	<10	<10	<10	<10	<20
Region Øst	Barley	<10	16	<10	<10	<20
Region Øst	Barley	59	<10	<10	<10	<20

Appendix Table 2. Concentrations of aflatoxin B1, B2, G1, G2, sum of aflatoxins, fumonisin B1, B2 and sum of fumonisins in individual samples of maize (13 samples) in 2015. All concentrations in µg/kg.

Region1	ID	Raw material	Afla B1	Afla B2	Afla G1	Afla G2	Sum Afla	Fum B1	Fum B2	FumB1+ FumB2
Region Midt	5659	Maize	0.13	<0.10	0.10	<0.10	<0.40	624	128	752
Region Midt	7056	Maize	0,13	<0.10	0.10	<0.10	<0.40	278	58	336
Region Midt	7051	Maize	0.13	<0.10	0.10	<0.10	<0.40	223	56	279
Region Midt	1303	Maize	<0.10	<0.10	<0.10	<0.10	<0.40	513	170	683
Region Midt	1309	Maize	<0.10	<0.10	<0.10	<0.10	<0.40	107	19	126
Region Midt	5256	Maize	<0.10	<0.10	<0.10	<0.10	<0.40	222	51	273
Region Midt	5268	Maize	0.13	<0.10	0.10	<0.10	<0.40	115	24	139
Region Øst	2253	Maize	100.4	7.3	0.10	<0.10	107.88	31	5	36
Region Øst	6538	Maize	97.40	17.4	0.10	<0.10	114.95	141	78	219
Region Sør og Vest	7416	Maize	<0.10	<0.10	<0.10	<0.10	<0.40	162	53	215
Region Sør og Vest	7417	Maize	<0.10	<0.10	<0.10	<0.10	<0.40	1 380	314	1 694
Region Sør og Vest	7418	Maize	<0.10	<0.10	<0.10	<0.10	<0.40	466	105	571
Region Sør og Vest	7419	Maize	0.10	<0.10	<0.10	<0.10	<0.40	8 750	3 540	12 290

Appendix Table 3. Concentrations of deoxynivalenol (DON), nivalenol (NIV), HT-2 toxin, 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 (30 samples) in 2015. All concentrations in µg/kg.

Complete feed for pigs									
Region	ID	Type of feed	DON	NIV	HT-2	T-2	HT-2+T2	ZEA	OTA
Region Midt	5249	Sow	22	<10	10	<10	<20	4.7	<0.1
Region Midt	5269	Sow	78	<10	16	<10	21	6.4	<0.05
Region Midt	1304	Sow	185	27	74	27	101	13.1	0.2
Region Midt	7058	Sow	175	64	68	25	93	11.0	0.14
Region Midt	5258	Piglets	193	12	38	15	53	49.6	0.18
Region Midt	3967	Piglets	96	31	42	15	57	22.2	0.24
Region Midt	5665	Growing pig	107	<10	68	20	88	3.4	<0.1
Region Midt	5661		162	116	82	33	115	11.3	0.18
Region Stor-Oslo	6388	Sow	22	<10	18	10	28	12.7	<0.05
Region Stor-Oslo	4287	Growing pig	179	48	55	16	71	7.2	0.26
Region Stor-Oslo	4289		136	<10	38	30	68	36.4	0.23
Region Øst	2250	Sow	60	<10	68	51	119	15.5	0.34
Region Øst	7808	Sow	92	<10	28	14	42	9.0	<0.1
Region Øst	6616	Sow	174	32	94	33	127	79.2	0.3
Region Øst	6659	Sow	83	35	66	16	82	4.3	0.3
Region Øst	2251	Piglets	67	<10	27	<10	32	217.2	0.29
Region Øst	7805	Piglets	73	10	35	<10	40	69.5	0.1
Region Øst	7806	Piglets	130	<10	21	<10	26	182.5	0.4
Region Øst	6661	Piglets	132	<10	21	<10	26	22.8	0.4
Region Øst	7479	Growing pig	88	19	51	20	71	<3	0.26
Region Øst	6660	Growing pig	156	11	50	19	69	6.0	<0.1
Region Øst	5423	Growing pig	25	14	17	<10	22	9.9	0.15
Region Øst	-		34	<10	80	60	140	14.1	0.1
Region Sør og Vest	7378	Sow	140	17	40	23	63	19.8	1.44
Region Sør og Vest	7631	Sow	235	16	43	10	53	67.0	0.6
Region Sør og Vest	7377	Piglets	67	<10	22	10	32	153.7	0.17
Region Sør og Vest	7542	Piglets	20	<10	13	<10	18	10.9	0.1
Region Sør og Vest	7376	Growing pig	106	13	63	24	87	1.5	0.11
Region Sør og Vest	7630	Growing pig	189	22	81	14	95	14.3	0.7
Region Sør og Vest	7412	Growing pig	289	46	66	31	97	22.3	0.4

Appendix Table 4. Concentrations of aflatoxin B1, B2, G1 and G2 in individual samples of complete feed for ruminants (38 samples) in 2015. All concentrations in µg/kg.

Complete feed for ruminants						
Region	**ID**	**Type of feed**	**Afla B1**	**Afla B2**	**Afla G1**	**Afla G2**
Region Nord	2898	Dairy cows	<0.1	<0.1	<0.1	<0.1
Region Nord	2897	Sheep	<0.1	<0.1	<0.1	<0.1
Region Midt	7050	Dairy cows	<0.75	<0.3	<0.6	<0.45
Region Midt	5267	Dairy cows	<0.75	<0.3	<0.6	<0.45
Region Midt	5259	Dairy cows	<0.75	<0.3	<0.6	<0.45
Region Midt	5664	Dairy cows	<0.1	<0.1	<0.1	<0.1
Region Midt	7049	Dairy cows	<0.75	<0.3	<0.6	<0.45
Region Midt	5250	Dairy cows, organic	<0.75	<0.3	<0.6	<0.45
Region Midt	5657	Dairy cows, organic	<0.75	<0.3	<0.6	<0.45
Region Midt	5266	Beef cattle	<0.75	<0.3	<0.6	<0.45
Region Midt	5663	Beef cattle	<0.1	<0.1	<0.1	<0.1
Region Midt	5656	Calf	<0.75	<0.3	<0.6	<0.45
Region Midt	7134		<0.75	<0.3	<0.6	<0.45
Region Midt	7136		<0.75	<0.3	<0.6	<0.45
Region Midt	7137		<0.75	<0.3	<0.6	<0.45
Region Stor-Oslo	6419	Beef cattle	<0.1	<0.1	<0.1	<0.1
Region Øst	5422	Dairy cows	<0.75	<0.3	<0.6	<0.45
Region Øst	7477	Dairy cows	<0.75	<0.3	<0.6	<0.45
Region Øst	7478	Dairy cows	<0.75	<0.3	<0.6	<0.45
Region Øst	6657	Dairy cows	<0.1	<0.1	<0.1	<0.1
Region Øst	6658	Dairy cows	<0.1	<0.1	<0.1	<0.1
Region Øst	1307	Dairy cows	<0.1	<0.1	<0.1	<0.1
Region Øst	6615	Dairy cows	<0.1	<0.1	<0.1	<0.1
Region Øst	6614	Dairy cows	<0.1	<0.1	<0.1	<0.1
Region Øst	6617	Dairy cows	<0.1	<0.1	<0.1	<0.1
Region Øst	5546	Dairy cows, organic	<0.1	<0.1	<0.1	<0.1
Region Øst	7476	Beef cattle	<0.75	<0.3	<0.6	<0.45
Region Øst	6613		<0.75	<0.3	<0.6	<0.45
Region Sør og Vest	6344	Dairy cows	<0.75	<0.3	<0.6	<0.45
Region Sør og Vest	7408	Dairy cows	<0.75	<0.3	<0.6	<0.45
Region Sør og Vest	7375	Dairy cows	<0.75	<0.3	<0.6	<0.45
Region Sør og Vest	7544	Dairy cows	<0.75	<0.3	<0.6	<0.45
Region Sør og Vest	3971	Dairy cows	<0.75	<0.3	<0.6	<0.45
Region Sør og Vest	3972	Dairy cows	<0.75	<0.3	<0.6	<0.45
Region Sør og Vest	7413	Dairy cows	<0.1	<0.1	<0.1	<0.1
Region Sør og Vest	7414	Dairy cows	<0.1	<0.1	<0.1	<0.1
Region Sør og Vest	7415	Beef cattle	<0.1	<0.1	<0.1	<0.1
Region Sør og Vest	7543	Sheep	<0.75	<0.3	<0.6	<0.45

Appendix Table 5. Concentrations of yeast, total mould, storage mould, deoxynivalenol (DON), nivalenol (NIV), HT-2 toxin, T-2 toxin and sum of HT-2 and T-2 toxin (HT-2+T-2) in complete farm-mixed, wet feed for pigs sampled in 2015.

Complete farm-mixed wet feed for pigs									
Region	**ID**	**Yeast cfu/g**	**Total mould cfu/g**	**Storage mould cfu/g**	**DON µg/kg**	**NIV µg/kg**	**HT-2 µg/kg**	**T-2 µg/kg**	**HT2+ T-2**
Region Midt	05275	150	<50	<50	<10	<10	<10	<10	<20
Region Midt	05273	500	1 500	1 500	34	<10	<10	<10	<20
Region Midt	05274	20 000	<50	<50	<10	<10	<10	<10	<20
Region Stor-Oslo	3211	2 800	730	550	13	<10	<10	<10	<20
Region Stor-Oslo	9451	5 000	60 000	60 000	10	<10	<10	<10	<20
Region Stor-Oslo	06278	6 400	350	350	<10	<10	<10	<10	<20
Region Stor-Oslo	6387	25 000	9 100	7 000	<10	<10	<10	<10	<20
Region Stor-Oslo	6386	68 000	17 000	14 500	<10	<10	<10	<10	<20
Region Øst	02288	<50	750	750	<10	<10	<10	<10	<20
Region Øst	06632	<50	<50	<50	16	<10	10	<10	<20
Region Øst	09489	<50	<50	<50	<10	<10	<10	<10	<20
Region Øst	06539	250	<50	<50	<10	<10	<10	<10	<20
Region Øst	05562	730 000	50 000	25 000	<10	<10	<10	<10	<20
Region Sør og Vest	07384	150	<50	<50	<10	<10	<10	<10	<20
Region Sør og Vest	07382	3 000	<50	<50	<10	<10	<10	<10	<20

Appendix Table 6. Concentrations of yeast, total mould, storage mould, deoxynivalenol (DON), nivalenol (NIV), HT-2 toxin, T-2 toxin and the sum of HT-2 and T-2 toxin (HT-2+T-2) in complete farm-mixed feed for ruminants sampled in 2015.

Complete farm-mixed feed for ruminants									
Region	**ID**	**Yeast cfu/g**	**Total mould cfu/g**	**Storage mould cfu/g**	**DON µg/kg**	**NIV µg/kg**	**HT-2 µg/kg**	**T-2 µg/kg**	**HT2+ T-2**
Region Midt	05271	2 500 000	10 000	10 000	38	<10	21	<10	26
Region Midt	05272	39 000 000	75 000	75 000	24	<10	19	<10	24
Region Midt	05270	74 000 000	<50	<50	<10	<10	<10	<10	<20
Region Stor-Oslo	4	180	<50	<50	12	<10	<10	<10	<20
Region Stor-Oslo	12	>4 000 000	70 000	70 000	<10	<10	21	<10	26
Region Øst		19 000 000	15 000	10 000	79	<10	22	<10	27
Region Øst	05497	44 000 000	250 000	20 000	56	20	26	<10	31
Region Sør og Vest	07383	<50	<50	<50	<10	<10	<10	<10	<20
Region Sør og Vest	8295	11 000	19 000	19 000	49	21	36	<10	41
Region Sør og Vest	8296	140 000	860	860	29	12	24	<10	29
Region Sør og Vest	07385	210 000	280 000	280 000	24	<10	<10	<10	<20
Region Sør og Vest		>4 000 000	<50	<50	<10	<10	<10	<10	<20
Region Sør og Vest		9 900 000	<50	<50	19	<10	<10	<10	<20
Region Sør og Vest		64 000 000	150 000	150 000	11	<10	<10	<10	<20

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