



The surveillance programme for feed and feed materials for terrestrial animals in Norway 2022 - Mycotoxins, fungi and bacteria



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The surveillance programme for feed and feed materials for terrestrial animals in Norway 2022 - Mycotoxins, fungi and bacteria

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Content

Summary	3
Sammendrag	4
Introduction	5
Aims	6
Materials and methods	6
Chemical analysis of oats, barley, rye, compound feed for pigs and farm-mixed feed for pigs	7
Quantitative determination of <i>Claviceps purpurea</i> in barley and rye	9
Quantitative determination of fungi in farm-mixed feed for pigs.....	9
Detection of <i>Salmonella</i> in farm-mixed feed for pigs.....	10
Statistical analysis	10
Results and discussion	11
Cereals.....	11
Feed.....	19
Conclusions	22
Feed materials.....	22
Feed.....	22
Acknowledgements	23
References	23
Appendix	25

Summary

The surveillance programme for feed and feed materials in 2022 included mycotoxins of possible concern in oats, barley, rye, maize, as well as in compound feed and farm-mixed feed for pigs. In addition, ergot (*Claviceps purpurea*) was determined in barley and rye, and various fungi and *Salmonella* were determined in the farm-mixed feed.

In oats, the lowest concentrations of deoxynivalenol (DON) was found since the annual surveillance started in 2002. However, the DON level was higher in region Midt than in South-Eastern Norway, probably related to a greater humidity during the growth season in region Midt compared to the drier season in South-Eastern Norway. The levels of T-2/HT-2 toxin were below the average concentrations recorded in the last decade, but one sample was above the national guidance level after correction for analytical uncertainty.

Insignificant levels of mycotoxins were found in barley. Ergot was found in some barley samples at very low levels with no significant correlation between ergot and ergot alkaloids.

In rye, ergot was detected in all samples, in several at very high levels. Ergot alkaloids were also significantly elevated in several samples. Correlation between ergot and ergot alkaloids was not statistically significant. As the samples were taken from the batches before the rye was cleansed for ergot, the results are not representative for rye at the market.

In maize, aflatoxins were detected in one out of eight samples with concentrations of aflatoxin B1, B2 and G1 detected at 18.2, 1.80 and 0.10 µg/kg, respectively. The sample did not exceed the maximum limit of aflatoxin B1 (20 µg/kg). The sample that contained aflatoxin was maize gluten, whereas the other samples were whole maize. Zearalenone (ZEA) was found in most samples, but all were below the guidance level. Trace concentration of ochratoxin A (OTA) was found in a single sample.

In compound feed for pig, DON was found in most samples, but all at insignificant concentrations. Other trichothecenes, as well as ZEA, fumonisins and OTA were not or barely detectable. Ergot alkaloids were found in some samples at low levels. The levels of analysed mycotoxins in farm-mixed feed were lower than in the samples of compound feed.

Storage moulds and yeasts were the most prevalent fungi in farm-mixed feed for pigs. The most dominating genus were *Penicillium* spp., detectable in 83 % of the samples. Yeasts were found in all samples. The levels of fungi indicate reduced hygienic quality in several of the samples. *Salmonella* was not found.

Sammen drag

Overvåkingsprogrammet for fôr og fôrmidler i 2022 omfattet mykotoksiner av potensiell betydning i havre, bygg, rug, mais, samt i kraftfôr og gårdsblandet fôr til gris. I tillegg ble bygg og rug undersøkt for meldrøye (*Claviceps purpurea*), og gårdsblandet fôr undersøkt for diverse sopp, samt *Salmonella*.

Konsentrasjonene av deoksynivalenol (DON) i havre var de laveste siden den årlige overvåkingen startet i 2002. Imidlertid var DON-nivået høyere i region Midt enn i regionene i Sørøst, trolig relatert til en fuktig vekstsesong i region Midt sammenlignet med en tørr sesong i regionene i Sørøst. Nivåene av T-2/HT-2 toksin var under gjennomsnittskonsentrasjonene fra det siste tiåret, men *en* havreprøve var over anbefalt grense etter korrigering for analytisk usikkerhet.

I bygg ble det funnet ubetydelige konsentrasjoner av mykotoksiner. Meldrøye ble funnet i noen prøver i svært lave nivåer. Det var ikke signifikant korrelasjon mellom meldrøye og meldrøyealkaloider.

I rug ble meldrøye påvist i alle prøver og i svært høye nivåer i flere prøver. Også meldrøyealkaloider var betydelig forhøyet i flere prøver. Korrelasjonen mellom meldrøye og meldrøyealkaloider var ikke statistisk signifikant. Ettersom prøvene ble samlet fra rugpartiene før rensing for meldrøye, er resultatene ikke representative for rug på markedet.

I mais ble aflatoksiner påvist i *en* av åtte undersøkte prøver med konsentrasjoner av aflatoksin B1, B2 og G1 på henholdsvis 18.2, 1.80 and 0.10 µg/kg. Prøven overskred ikke maksimalgrensen for aflatoksin B1 (20 µg/kg). Prøven som inneholdt aflatoksiner var av maisgluten, mens de andre prøvene var av hel mais. Zearalenon (ZEA) ble funnet i de fleste prøvene, alle under veiledende grense, og spor av okratoksin A (OTA) ble funnet i *en* prøve.

I kraftfôr til gris ble det funnet DON i de fleste prøvene, alle i ubetydelige konsentrasjoner. Andre trichothecener, samt ZEA, fumonisiner og OTA ble ikke, eller nesten ikke, påvist. Nivåene av analyserte mykotoksiner i gårdsblandet fôr var enda lavere enn i prøvene av kraftfôr.

Det meste av soppen i gårdsblandet fôr til gris var lagringsmuggsopp og gjærsopp. Den mest dominerende slekten var *Penicillium* spp., som ble påvist i 83 % av prøvene. Gjærsopp ble funnet i alle prøvene. Nivået av sopp indikerer redusert hygienisk kvalitet i flere av prøvene. *Salmonella* ble ikke påvist.

Introduction

The annual surveillance programme on mycotoxins and microorganisms in feed and feed materials is a collaboration between the Norwegian Food Safety Authority (NFSA) and the Norwegian Veterinary Institute (NVI). NFSA decides the scope of the programme based on scientific advice from NVI, with NFSA responsible for collecting the samples, NVI for analysing and reporting of the results, and finally NFSA for result management. The agents for analyses usually consists of important mycotoxins and fungi (moulds, yeasts and ergot), in some years also selected bacteria. The programme gives good basis for assessments of feed quality, the impact of animal health and human exposure via animal products.

Fungi in cereals may be differentiated into field and storage fungi. Field fungi invade the seeds before harvest, and may affect the appearance and quality of seed or grain. Common field fungi in Norwegian cereal grain include mould species of the genera *Fusarium*, *Alternaria*, *Microdochium*, *Cladosporium*, *Acremonium*, *Epicoccum*, *Phoma* and more. In addition, *Claviceps purpurea* (ergot) is a field fungus [1]. Storage fungi usually occur in small amounts before harvest. However, under improper storage conditions, storage fungi can grow rapidly leading to significant problems. The most common storage fungi are *Penicillium*, *Aspergillus* and Mucorales. In addition, yeasts can occur in variable amounts among field and storage fungi [1].

Fusarium species are the most important mycotoxin-producing field fungi. They produce important mycotoxins such as the trichothecenes deoxynivalenol (DON), T-2 toxin (T-2) and HT-2 toxin (HT-2), as well as zearalenone (ZEA) [1].

Two decades of surveillance in Norwegian cereals have shown that DON can occur in high concentrations, particularly in oats and wheat. DON is hazardous to health if ingested by animals and humans [1]. Well-documented gastrointestinal disorders of DON exposure are reduced feed intake and stunted growth rate in pigs. In addition, DON impairs the immune system. T-2 and HT-2 are usually present in levels of concern only in oats and oat products. They have similar but potentially stronger toxic effects than DON, in causing gastrointestinal lesions as well as immune suppression [1]. Based on the limited available surveillance data, the oestrogenic mycotoxin ZEA produced by the same *Fusarium* species as DON, is usually present at insignificant levels in Norwegian cereals [1].

Data on the occurrence of the emerging mycotoxins ergot alkaloids are of considerable interest in EU [2]. They show moderately acute neurotoxic effects, inhibition of blood circulation and interference of hormone levels. Ergot alkaloids produced by *Claviceps purpurea* are found mainly in rye, but may also occur in other cereal species - usually more in barley than oats [3-7].

Species of genera *Penicillium* and *Aspergillus* are the most important mycotoxin-producing storage fungi. *Penicillium* species generally grow and produce mycotoxins at lower temperatures than species of *Aspergillus*, and are therefore of main concern under the Norwegian storage conditions [1].

Ochratoxin A (OTA) is an important mycotoxin produced by several species of both *Penicillium* and *Aspergillus*. The most prominent adverse effect of OTA in livestock is nephrotoxicity in pigs. It may also suppress the immune response and growth performance [1]. As far as we

know, OTA has not caused problems for Norwegian husbandry. Nonetheless, active surveillance of OTA is important, particularly because of imported feed ingredients [1].

In addition, aflatoxins produced by some *Aspergillus* species may occur in imported feed ingredients [1]. These carcinogenic and liver toxic compounds must remain at low levels to minimise human health risks via consumption of animal products as well as to ensure animal health. An active metabolite of aflatoxins secreted into the milk, can result in human exposure via dairy products.

Aims

The aims of the programme on surveillance of feed and feed materials for terrestrial animals in Norway are to document compliance with the legislation on the occurrence of important mycotoxins and selected microorganisms, primarily fungi. The data are used to assess adverse animal health risks related to these agents in feed and to human exposure of transmissible agents via animal products.

Materials and methods

In 2022, the surveillance programme for feed consisted of the following samples shown in Table 1.

Table 1. Samples in the surveillance programme for feed 2022.

Matrix	Planned	Sampled and analysed	Analyses
Oats	45	45	Trichothecenes, zearalenone and ergot alkaloids.
Barley	45	47	<i>Claviceps purpurea</i> , trichothecenes, zearalenone and ergot alkaloids.
Rye	20	14	<i>Claviceps purpurea</i> and ergot alkaloids.
Maize/maize products	15	8	Aflatoxins, ochratoxin A and zearalenone.
Complete compound feed for pigs	20	20	Trichothecenes, zearalenone, fumonisins, ergot alkaloids and ochratoxin A.
Farm-mixed feed for pigs	25	12	Mould and yeasts, <i>Salmonella</i> , trichothecenes, zearalenone, ergot alkaloids and ochratoxin A.

Oats, barley and rye from mills in grain production areas were sampled during autumn. Batches of imported maize from third countries, compound feed for pigs from feed industries and farm-mixed feed for pigs were sampled throughout the year. To ensure samples were representative, sampling followed EU Regulation 152/2009.

Chemical analysis of oats, barley, rye, compound feed for pigs and farm-mixed feed for pigs

The multi-mycotoxin liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used for the simultaneous determination of mycotoxins in oats, barley, rye, compound feed for pigs and farm-mixed feed for pigs. The method was validated 'in house' in order to ensure the quality and reliability of collected data. Performance parameters assessed were linearity, selectivity, limit of detection (LOD) and limit of quantification (LOQ). According to the validation data, considerable matrix effects varying from 10 to 98 % were demonstrated for all selected mycotoxins and matrices. Reasonable levels of signal suppression or signal enhancement (70 - 120 %) were achieved for only 15 % of the targeted mycotoxins. Therefore, in order to improve the accuracy of the method, stable-isotope labelled internal standards (IS) were introduced for ten of the analysed mycotoxins including deoxynivalenol (DON), its' related compounds 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON) and DON-3-glucoside (DON-3-G), as well as nivalenol (NIV), HT-2, T-2, fumonisin B1 (FB1), zearalenone (ZEA) and ochratoxin A (OTA). For the quantitative analysis of ergot alkaloids, matrix-matched calibrations were prepared for each matrix. Statistics from proficiency tests provided for the national reference laboratories (NRLs) and appointed official control laboratories (OCLs) confirmed the applicability of this approach.

The accuracy of the method was assessed by determining recovery from spiking experiments and the expanded measurement uncertainty ($U'(\%) = 2 \times (\text{mean}_{\text{bias}}^2 + \text{SD.P}_{\text{bias}}^2 + \text{RSD}_{\text{rW}}^2)^{1/2}$), where $\text{mean}_{\text{bias}}$ is the mean of the differences between the measured values and the true value, $\text{SD.P}_{\text{bias}}$ is the standard deviation of the population of the bias values, and where RSD_{rW} is the relative standard deviation of within-laboratory reproducibility (Table 2). The LODs of the targeted mycotoxins were determined by empirical methods, where blank samples containing known concentrations of targeted analytes were repeatedly analysed, so the minimum level of each analyte could be detected.

Table 2. Performance validation parameters for multi-analyte mycotoxin LC-MS/MS method.

Toxin	LOD, $\mu\text{g}/\text{kg}$	Expanded Measurement Uncertainty (%)				Recovery \pm SD (%)			
		Oats	Barley	Rye	Pig feed	Oats	Barley	Rye	Pig feed
eoxynivalenol	17	45	45	-	64	78 \pm 4	84 \pm 12	-	102 \pm 15
DON-3-glucoside	8	42	38	-	42	80 \pm 4	83 \pm 5	-	85 \pm 11
3-Acetyl-DON	25	34	32	-	33	85 \pm 4	88 \pm 7	-	95 \pm 11
15-Acetyl-DON	46	26	22	-	12	104 \pm 9	109 \pm 5	-	99 \pm 4
Nivalenol	13	57	47	-	43	73 \pm 4	81 \pm 9	-	87 \pm 11
T-2 toxin	25	45	43	-	45	75 \pm 4	80 \pm 5	-	91 \pm 15
HT-2 toxin	37	42	48	-	40	80 \pm 7	79 \pm 7	-	98 \pm 17
Fumonisin B1	32	28	21	-	48	107 \pm 9	106 \pm 6	-	95 \pm 16
Fumonisin B2	14	36	44	-	24	114 \pm 9	117 \pm 11	-	94 \pm 7
Zearalenone	14	60	49	-	55	71 \pm 5	76 \pm 4	-	86 \pm 16
Ochratoxin A	24	20	26	-	33	96 \pm 7	107 \pm 5	-	86 \pm 8
Ergonovine*	0.3	26	37	44	23	109 \pm 7	117 \pm 6	121 \pm 4	107 \pm 7
Ergosine*	11	48	41	44	35	123 \pm 5	120 \pm 2	120 \pm 5	113 \pm 9
Ergotamine*	14	51	53	39	44	123 \pm 8	126 \pm 4	116 \pm 7	115 \pm 13
Ergocornine*	25	38	46	32	34	119 \pm 3	122 \pm 5	113 \pm 4	110 \pm 10
α -Ergocryptine*	8	43	34	38	30	121 \pm 3	117 \pm 2	114 \pm 3	108 \pm 9
Ergocristine*	18	46	35	34	37	123 \pm 3	116 \pm 5	115 \pm 4	109 \pm 12

* Validation data cover ergot alkaloids and their corresponding -inine epimers

Samples were grinded to fine powder and a subsample of 2.5 g (\pm 0.2 %) was weighed in. The extraction methodology was based on a two-step extraction (MeCN:H₂O:HCOOH, 80:19.9:0.1, v/v/v and MeCN:H₂O:HCOOH, 20:79.9:0.1, v/v/v) in order to improve the extraction efficiency with respect to polar and non-polar compounds. Extracts were centrifuged, filtered and were ready for instrumental analysis.

The LC-MS/MS analyses were performed on an Agilent Triple Quadrupole LC-MS system (1290-6470), equipped with an AJS electrospray ionization (ESI) while the Agilent MassHunter workstation software was used for data acquisition and quantitative analysis. 2 μL of sample extract was injected into the LC system and analytes were separated on a Kinetex F5 100 Å column (100 x 2.1 mm), equipped with a precolumn, under a constant flow of 0,25 mL/min. Gradient elution was performed with a 5 mM ammonium acetate/1% acetic acid aqueous mobile phase and methanol to achieve optimal separation. Due to differences in the nature of each compound, the Triple Quadrupole was operated in both positive and negative ionization mode for optimal sensitivity. Identification of target mycotoxins was performed using three compound specific MRM transitions.

Chemical analysis of maize

In 2022 the maize samples were sent to Premier Analytical Services (PAS), England for mycotoxin analyses because of a lack of validation of the in house method for analysis of aflatoxins and ochratoxin in relation to the NVI's laboratory moving from Oslo to Ås. The PAS laboratory is accredited to United Kingdom Accreditation Service (UKAS) 17025 standards. Accreditation by UKAS demonstrates competence, impartiality and performance capability of the laboratory. The internationally recognised standard for the competence of laboratories is ISO17025 and is the standard against which UKAS uses to accredit laboratories. All methods accredited by UKAS are fully assessed for validity of use for each sample matrix. This validation covers specificity, linearity, sensitivity, repeatability, reproducibility, robustness and fitness for purpose in terms of regulatory compliance.

The analyses are conducted with a spiked sample, i.e. to each sample matrix, on each day, a known amount of toxin is added prior to extraction, clean up and detection. These samples are used to assess recovery, and recoveries of 70-110% are classed as valid. Spiked samples are also used for quantification, thus making all results recovery corrected.

Aflatoxins: B1, B2, G1 and G2 were determined using immunoaffinity clean-up and high performance liquid chromatography with fluorescence detection. PAS uses the UKAS accredited method BA-TM-10 for the analysis of aflatoxin B1, B2, G1 and G2. Limit of Quantification: 0.1 µg/kg for each toxin.

Zearalenone was determined using immunoaffinity clean-up and high performance liquid chromatography with fluorescence detection. PAS uses the UKAS accredited method BA-TM-11 for the analysis of zearalenone. Limit of Quantification: 3 µg/kg.

Ochratoxin A was determined using immunoaffinity clean-up and high performance liquid chromatography with fluorescence detection. PAS uses the UKAS accredited method BA-TM-15 for the analysis of ochratoxin A. Limit of Quantification: 0.1 µg/kg.

Quantitative determination of *Claviceps purpurea* in barley and rye

Claviceps purpurea sclerotia in grams per kg cereal were calculated according to the method described by Vrålstad *et al.* [8]. The weighed sample was spread over a large light surface for visual inspection. Detected sclerotia of *C. purpurea* were picked out and weighed separately.

Quantitative determination of fungi in farm-mixed feed for pigs

Quantitative determinations of moulds and yeasts in farm-mixed feed for pigs were performed by using NMKL method No 98 and using Dichloran 18 % glycerol agar (DG18) as growth medium. In addition, a qualitative determination of the composition of the mycoflora was performed by identification and counting *Penicillium*, *Aspergillus* and Mucorales separately. The detection limit was 50 colony-forming units per gram (cfu/g).

Detection of *Salmonella* in farm-mixed feed for pigs

For the detection of *Salmonella* in farm-mixed feed for pigs, NS-EN ISO 6579-1:2017 was used. Briefly, 25 g sample was enriched in buffered peptone water (BPW-ISO) followed by selective enrichment in Rappaport-Vassiliadis broth with soya (RVS) and Muller-Kauffman tetrathionate-novobiocin broth (MKTTn) with subsequent plating on Xylose Lysine Deoxyholate (XLD) agar and Brilliant Green agar (BGA). Typical and suspicious colonies were pure-cultured and further identified using Maldi-TOF.

Statistical analysis

Descriptive statistics followed by One-way Anova were used to determine significance in statistical differences between groups for variables that were measured quantitatively. To investigate possible linear correlation between two variables in the same feed type, scatter plots and Pearson correlations with p-values were determined.

Half detection limits (half quantification limits for toxins in maize) specific to a variable were used for calculation purposes when levels were not detectable.

Results and discussion

Cereals

Mycotoxins in oats

In oats in 2022, DON was detectable in 80 % of the samples, and with a mean concentration of 83 µg/kg, DON was detected at the lowest concentrations since 2002 (Table 3; Figure 1). All samples had levels far below the limit for DON recommended by EU and Norway (8000 µg/kg) [9, 10].

The DON-related compounds included in the analysis of oats were the acetylated precursor compounds (3-Ac-DON and 15-Ac-DON) and DON-3-glucoside (DON-3-G). DON-3-G was detectable in 29 % of the samples, whereas 3-Ac-DON were hardly detected and 15-Ac-DON was not detected. DON and DON-3-G were significantly positively correlated (Figure 2).

Table 3. Concentrations (µg/kg) of deoxynivalenol (DON), 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON), DON-3-glucoside (DON-3-G), T-2 and HT-2 toxin and the sum of T-2/HT-2, nivalenol (NIV), zearalenone (ZEA), ochratoxin A (OTA) and sum ergot alkaloids (ΣErg alk) in oats (N = 45) sampled in Norway in 2022.

	DON	3-Ac-DON	15-Ac-DON	DON-3-G	T-2	HT-2	T-2+HT-2	NIV	ZEA	OTA	ΣErg alk
Mean	83	<25	<46	15	46	99	146	13	<14	<24	<76
Median	37	<25	<46	<8	28	62	88	<13	<14	<24	<76
Minimum	<17	<25	<46	<8	<25	<37	<62	<13	<14	<24	<76
Maximum	459	45	<46	126	369	622	991	65	<14	<24	484
SD*	108	33	0	27	64	109	171	15	0	0	68
% samples >dl*	80	7	0	29	51	67	64	20	0	0	4
% samples >gv*	0						4		0		

* SD = Standard Deviation, >dl = above detection limits, >gv = above guidance values.

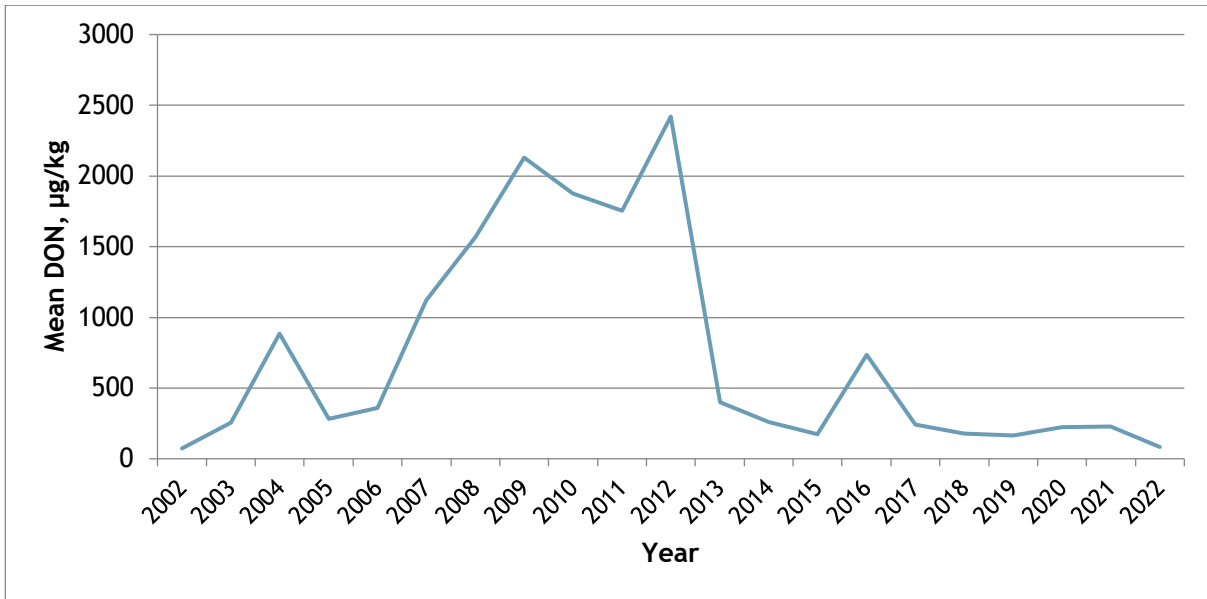


Figure 1. Mean concentration of deoxynivalenol (DON) in 30-60 samples of oats per year in the Norwegian surveillance programme for feed.

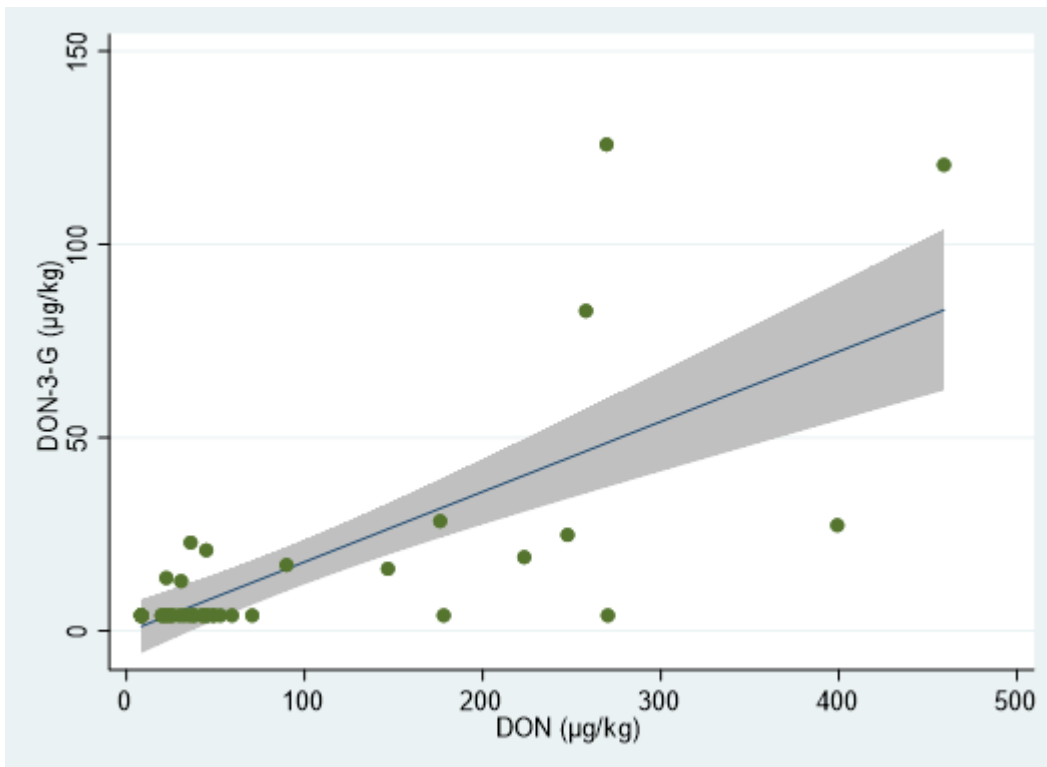


Figure 2. The Pearson correlation between deoxynivalenol (DON) and DON-3-glucoside (DON-3-G) in oats (N=45) 2022 was $r=0.73$, $p<0.0001$. A regression line with 95 % confidence interval fitted to the points allows predictions of levels of DON-3-G given the level of DON detected and vice versa.

T-2 and HT-2 were present in 64 % of the oat samples. Two samples (4 %) had combined sum T-2+HT-2 concentrations numerically above the guidance level of 500 µg/kg in EU and Norway [9, 11] (Table 3). However, one of the samples did not exceed the regulatory limit after factoring in the uncertainty of the analytical method. Thus, one sample exceeded the guidance level after discounting uncertainty. The mean concentration of T-2+HT-2 was below the average of corresponding levels from the last decade (Figure 3). T-2 and HT-2 were highly correlated in oats, with an average concentration of HT-2 about twice that of T-2 (Figure 4), which is similar to previous years [3-7, 12].

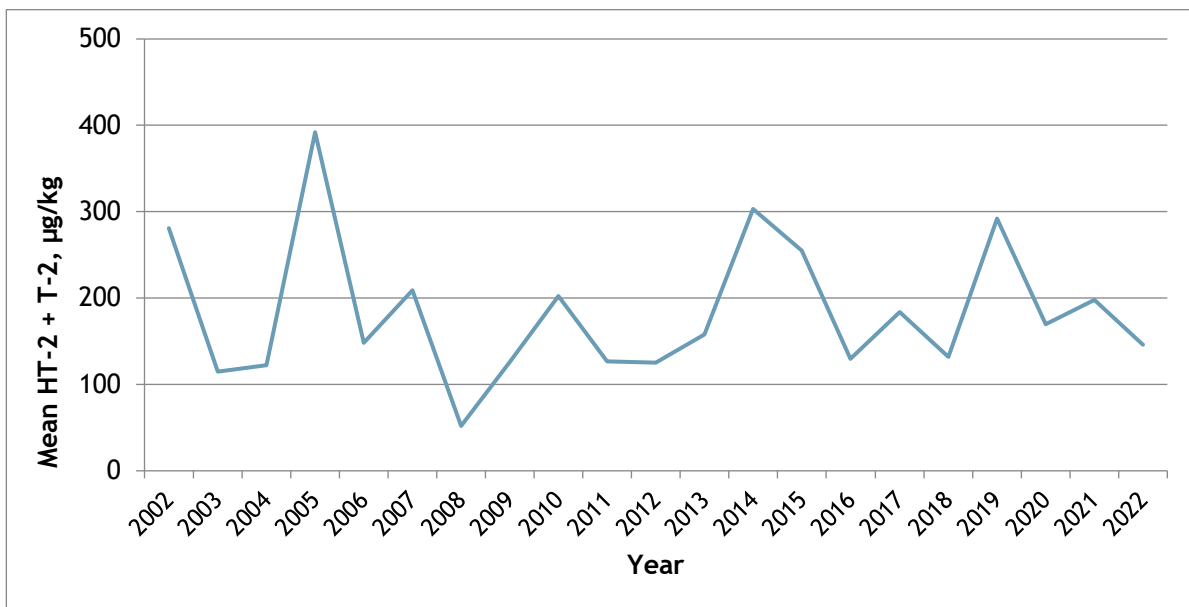


Figure 3. Mean concentration of the sum of T-2 toxin and HT-2 toxin in 30-60 samples of oats per year in the Norwegian surveillance programme for feed.

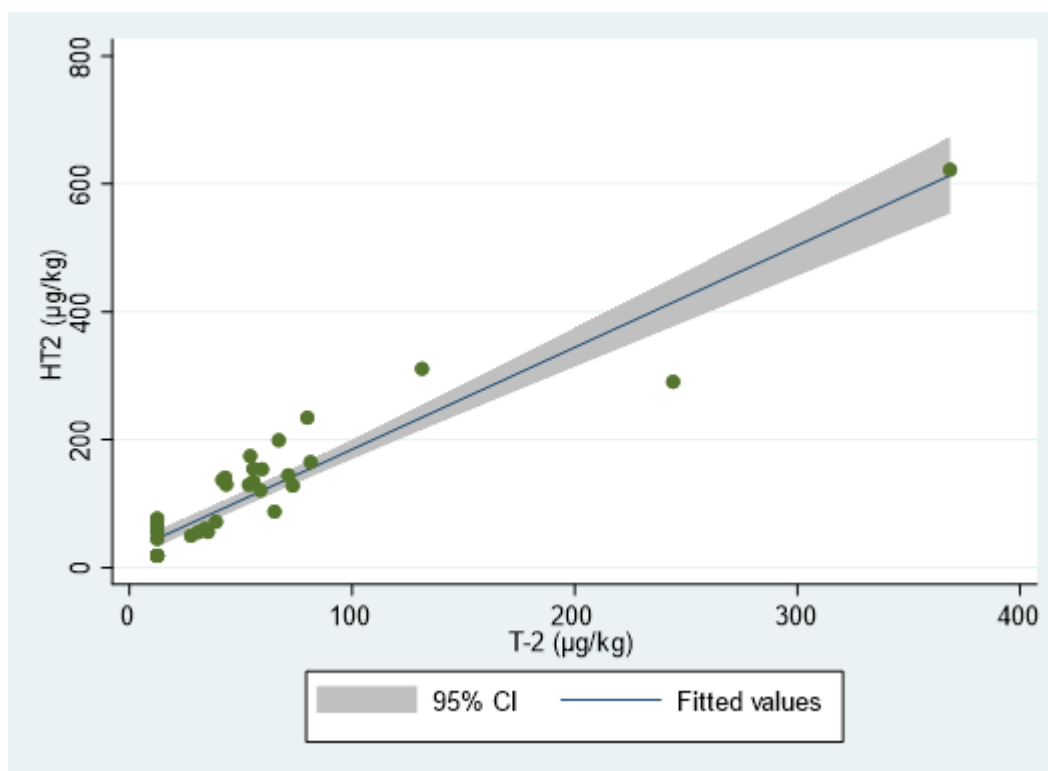


Figure 4. The Pearson correlation between T-2 toxin and HT-2 toxin in oats (N=45) 2022 was $r=0.94$, $p<0.0001$. A regression line with 95 % confidence interval fitted to the points allows predictions of levels of HT-2 toxin given the level of T-2 toxin detected and vice versa.

NIV was detected at low level in 20 % of the samples (Table 3). Ergot alkaloids were detected in two samples (4 %). ZEA or OTA were not detected.

Mycotoxins in oats were analysed for regional differences (Table 4). Significantly higher level of DON was found in region Midt than in regions Øst and Stor-Oslo (South-Eastern Norway). No significant regional differences were found for T-2/HT-2 or NIV.

Table 4. Survey on regional differences amongst Sør/Vest (Agder, Rogaland, Vestland), Øst (Buskerud, Vestfold, Telemark, Innlandet), Stor-Oslo (Akershus, Oslo, Østfold) and Midt (Trøndelag, Møre, Romsdal) on deoxynivalenol (DON), sum of T-2 and HT-2 toxin, and nivalenol (NIV)) in oats (N = 45) sampled in Norway in 2022. Variables that were significantly different between regions are indicated by an * ($p<0.05$).

Region		DON*	T-2+ HT-2	NIV
Sør-Vest n=1	Mean	<17	70	<13
	St. dev.			
Øst n=15	Mean	50	194	<13
	St. dev.	98	252	13
Stor-Oslo n=21	Mean	66	149	16
	St. dev.	70	117	17
Midt n=8	Mean	196*	57	<13
	St. dev.	150	54	15

The weather during the growing season is usually a key factor for the *Fusarium* and mycotoxin contents of cereal grains. Among the mycotoxins, the influence of the weather is particularly studied for DON. The level of precipitation and humidity during flowering (usually in July), as well as precipitation up to harvest in autumn are considered particularly important in influencing *Fusarium* and DON levels, while the temperature may also play a role [1].

July 2022 in South-Eastern Norway (Regions Øst and Stor-Oslo) was very dry with average temperature, whereas the Midt region had more rain and somewhat lower temperature than normal [13]. August in South-Eastern Norway had precipitation less than average and was relatively warmer than usual, while September experienced normal precipitation and temperature. In the Midt region, August and September had more rain than normal while having temperatures in the normal range. The weather in region Sør-Vest is not commented here as only a single oat sample was collected. It is likely that the relatively humid weather conditions in the Midt region during the growing season have contributed to higher concentrations of DON in oats from this region. However, the DON level measured in oats from this region did not constitute a risk related to the guidance level.

Claviceps purpurea and mycotoxins in barley

The pattern of lower concentrations of trichothecenes in barley compared with oats has been the same year after year [3-7, 12]. Table 5 shows the 2022 results of trichothecenes, ZEA and OTA in barley. Rather insignificant levels of trichothecenes and no detection of ZEA and OTA were found.

Claviceps purpurea (ergot) and ergot alkaloids were also determined in barley (Table 6) as they are usually more present in this species than in oats. *C. purpurea* was detected in 32 % of the samples. The ergot had an overall mean level of 4 mg/kg and a maximum of 55 mg/kg. Thus, all samples were far below the legislated maximum concentration of 1000 mg/kg [9]. The occurrence of ergot was somewhat lower than the corresponding levels observed in barley in 2019-2021 [3-4, 12]. Much of the ergot sclerotia in barley were small in size, indicating they had been growing on other grass and randomly contaminating barley.

The ergot alkaloids were present at low levels in barley with a maximum concentration of sum alkaloids 264 µg/kg. The alkaloids were detectable in 11 % of the samples.

Ergot alkaloids have been included in the analysis repertoire of barley since 2016. They have been only sporadically present, but in some samples of significant concentrations: Except in 2018 where none was detectable, maximum sum alkaloids were between 2200 and 3000 µg/kg in 2016, -17 and -19 [4-7], which are levels of possible animal health concern if barley from these batches were used as major feed ingredients [6]. In 2020-2022, the maximum levels have been lower [12]. Knowledge on possible influence of climate or weather conditions on occurrence of ergot and ergot alkaloids is lacking.

Table 5. Concentrations ($\mu\text{g}/\text{kg}$) of deoxynivalenol (DON), 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON), DON-3-glucoside (DON-3-G), T-2 and HT-2 toxin and the sum of T-2/HT-2, nivalenol (NIV), zearalenone (ZEA) and ochratoxin A (OTA) in barley (N = 47) sampled in Norway in 2022.

	DON	3-Ac-DON	15-Ac-DON	DON-3-G	T-2	HT-2	T-2+HT-2	NIV	ZEA	OTA
Mean	24	<25	<46	9	<25	<37	<62	<13	<14	<24
Median	<17	<25	<46	<8	<25	<37	<62	<13	<14	<24
Minimum	<17	<25	<46	<8	<25	<37	<62	<13	<14	<24
Maximum	217	<25	<46	93	80	38	118	46	<14	<24
SD*	35	0	0	16	10	3	13	8	0	0
% samples >dl*	40	0	0	17	4	2	2	9	0	0
% samples >gv*	0						0		0	0

* SD = Standard Deviation, >dl = above detection limits, >gv = above guidance values.

Table 6. Concentrations of *Claviceps purpurea* sclerotia (mg/kg) and ergot toxins ($\mu\text{g}/\text{kg}$) consisting of ergotamine/ergotaminine, ergocornine/ergocorninine, alpha-ergocryptine/alpha-ergocryptinine, ergocristine/ergocristinine and sum ergot alkaloids in barley (N = 47) sampled in Norway in 2022.

	C. purpurea sclerotia	Ergo-novine/-inine	Ergo-sine/-inine	Ergot-amine/-inine	Ergo-cornine/-inine	α -Ergo-cryptine/-inine	Ergo-cristine/-inine	Σ Ergot alkaloids
Mean	4	<0.3	<11	<14	<26	<8	<18	<76
Median	0	<0.3	<11	<14	<26	<8	<18	<76
Minimum	0	<0.3	<11	<14	<26	<8	<18	<76
Maximum	55	0.6	48	<14	51	29	216	264
SD*	11	0.07	9	0	6	4	33	41
% samples >dl*	32	2	15	0	2	4	13	11

* SD = Standard Deviation, >dl = above detection limits.

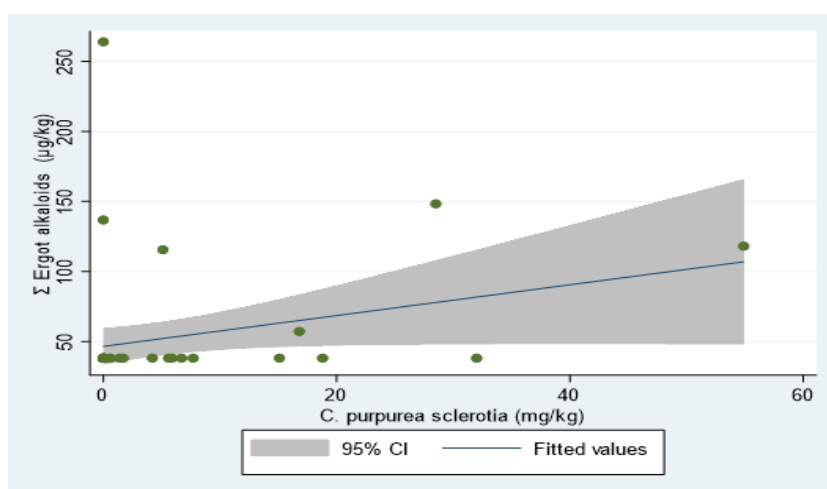


Figure 5. The Pearson correlation between *Claviceps purpurea* and sum of ergot alkaloids in samples of barley (n = 47) 2022 was $r=0.28$, $p=0.060$. A regression line fitted to the points with 95 % confidence interval is shown.

The correlation between the ergot and the ergot alkaloids was not statistically significant (Figure 5). Except for a significant correlation between ergot and ergot alkaloids in barley in 2021, lack of correlations between ergot and alkaloids were found in previous surveys in Norway, in barley in 2019 and 2020, and in rye and wheat in 2016 and 2017 [3-4, 12, 14-15]. The low or lack of correlation indicate interactions with different variables in production of the alkaloids by ergot fungi.

Mycotoxins and ergot in barley were analysed for regional differences (Table 7), without finding significant differences.

Table 7. Survey on regional differences amongst Sør/Vest (Agder, Rogaland, Vestland), Øst (Buskerud, Vestfold, Telemark, Innlandet), Stor-Oslo (Akershus, Oslo, Østfold) and Midt (Trøndelag, Møre, Romsdal) on deoxynivalenol (DON), sum of T-2 and HT-2 toxin, nivalenol (NIV), zearalenone (ZEA) and sum of ergot alkaloider (Σ Erg alk); all toxin concentrations $\mu\text{g}/\text{kg}$ and *Claviceps purpurea* (mg/kg) in barley (N = 47) sampled in Norway in 2022. There were no regional difference at the $p=0.05$ statistical significance for all variables.

Region		DON	T-2 + HT-2	NIV	Σ Erg alk.	C. pur.
Sør og Vest n=4	Mean	<17	<62	<13	<76	4
	St. dev.	0	0	0	10	8
Øst n=13	Mean	20	<62	<13	<76	7
	St. dev.	30	7	0	38	15
Stor-Oslo n=20	Mean	36	<62	<13	<76	3
	St. dev.	45	0	11	0	8
Midt n=10	Mean	<17	<62	<13	<76	3
	St. dev.	14	27	0	76	9

Claviceps purpurea and mycotoxins in rye

Claviceps purpurea was detected in all rye samples with several at very high levels (Table 8). Also the concentrations of ergot alkaloids were significantly elevated in several samples with one sample having sum ergot alkaloid concentration at 13 mg/kg. Correlation between ergot and ergot alkaloids was near statistical significant ($p=0.055$; Figure 6). The samples were taken from the batches before the rye was ergot cleansed. Thus, the results are not representative for rye at the market.

Too few samples of rye were collected to include studies of regional variations.

Table 8. Occurrence of *Claviceps purpurea* sclerotia (mg/kg) and ergot toxins ($\mu\text{g/kg}$) consisting of ergotamine/ergotaminine, ergocornine/ergocorninine, alpha-ergocryptine/alpha-ergocryptinine, ergocristine/ergocristinine and sum ergot alkaloids in rye sampled in Norway in 2022.

	<i>C. purpurea</i> sclerotia N=14	Ergo- novine/ -inine N=12	Ergo- sine/ -inine N=12	Ergot- amine/ -inine N=12	Ergo- cornine/ -inine N=12	α -Ergo- cryptine/ -inine N=12	Ergo- cristine /-inine N=12	Σ Ergot alkaloids N=12
Mean	2 693	29	209	350	159	371	627	1 740
Median	2 455	11	73	158	51	201	224	748
Minimum	26	<0.3	<11	<14	<25	<8	<18	<76
Maximum	7 910	227	1 460	2 560	1 260	2 240	5 420	13 160
SD*	2 507	63	406	713	350	620	1 521	3 630
% samples >dl*	100	75	83	75	58	75	75	83

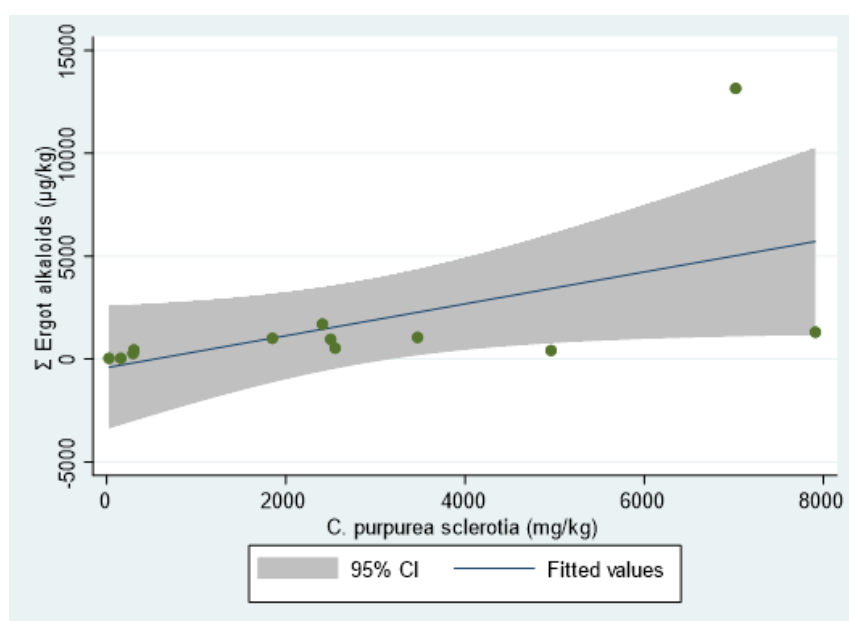


Figure 6. The Pearson correlation between *Claviceps purpurea* and sum of ergot alkaloids in samples of rye ($n=12$) 2022 was $r=0.57$, $p=0.055$. A regression line fitted to the points with 95 % confidence interval is shown.

Aflatoxins in maize

Aflatoxins were detectable in one of eight analysed maize samples, with concentrations of aflatoxin B1, B2 and G1 detected at 18.2, 1.80 and 0.10 $\mu\text{g/kg}$, respectively (Table 9). The sample did not exceed the maximum limit of aflatoxin B1 (20 $\mu\text{g/kg}$) [16]. The sample that contained aflatoxins was maize gluten, whereas the other samples were whole maize.

In 2022 like in 2021, also OTA and ZEA were analysed in maize (Table 8). Trace concentrations of OTA were detected in one sample at far below the guidance level of 250 $\mu\text{g/kg}$ [9]. ZEA was detected in most samples up to 206 $\mu\text{g/kg}$, which were far below the guidance level of 3000 $\mu\text{g/kg}$ [9].

Table 9. Concentrations ($\mu\text{g}/\text{kg}$) of aflatoxins (B1, B2, G1, G2), ochratoxin A (OTA) and zearalenone (ZEA) in maize (N = 8) sampled in Norway in 2022.

	Afla B1	Afla B2	Afla G1	Afla G2	OTA	ZEA
Mean	2.32	0.27	<0.10	<0.10	<0.10	64
Median	<0.10	<0.10	<0.10	<0.10	<0.10	19
Minimum	<0.10	<0.10	<0.10	<0.10	<0.10	<3
Maximum	18.20	1.80	0.10	<0.10	0.30	206
SD*	6.42	0.62	0.02	0	0.09	82
% samples >dl*	13	13	13	0	13	75
% samples >gv*	0				0	0

Feed

Feed for pigs

Samples of complete compound feed for pigs were analysed for trichothecenes, ZEA, OTA, fumonisins and ergot alkaloids.

The results on mycotoxins in complete compound feed for pigs in Table 10 show that DON was detected in 80 % of the samples but all at low levels well below the guidance level of DON for pig feed in Norway (500 $\mu\text{g}/\text{kg}$) [9]. Co-occurrence of DON with DON-related compounds were mostly undetectable, with only trace amounts of DON-3-G in some samples. Related compounds of DON can be an additional factor to the total DON exposure and EFSA considers their toxic effects to be similar to that of DON [17].

T-2 and HT-2 were hardly present in samples of the compound feed for pigs and their concentrations did not exceed the guidance level (250 $\mu\text{g}/\text{kg}$) in any sample [9, 11]. NIV and OTA were not detected in any sample. However, it has to be commented that the detection limit for OTA in the multi-toxin method is higher (24 $\mu\text{g}/\text{kg}$) than the guidance level of OTA in feed for pigs at 10 $\mu\text{g}/\text{kg}$ [9]. ZEA and fumonisins were present at trace concentrations in single samples.

Ergot alkaloids were present in several samples of the compound feed (Table 11). All concentrations were considered not to constitute any health risk for the pigs.

Table 10. Concentrations ($\mu\text{g}/\text{kg}$) of deoxynivalenol (DON), DON-3-glucoside (DON-3-G), sum of T-2 and HT-2 toxin, nivalenol (NIV), zearalenone (ZEA) fumonisin B1 and B2, and ochratoxin A (OTA) in complete compound feed for pigs ($N = 20$) sampled in Norway in 2022.

	DON	DON-3-G	T-2 + HT-2	NIV	ZEA	FUMB1	FUMB2	OTA
Mean	48	<8	<62	<13	<14	<32	<14	<24
Median	36	<8	<62	<13	<14	<32	<14	<24
Minimum	<17	<8	<62	<13	<14	<32	<14	<24
Maximum	128	32	<62	<13	16	62	16	<24
SD*	36	9	4	0	2	10	3	0
% samples >dl*	80	30	5	0	5	5	10	0
% samples >gv*	0		0		0			0

* SD = Standard Deviation, >dl = above detection limits, >gv = above guidance values.

Table 11. Concentrations of ergot toxins ($\mu\text{g}/\text{kg}$) consisting of ergotamine/ergotaminine, ergocornine/ergocorninine, alpha-ergocryptine/alpha-ergocryptinine, ergocristine/ergocristinine and sum ergot alkaloids in complete compound feed for pigs ($N = 20$) sampled in Norway in 2022.

	Ergo- novine/- inine	Ergo- sine/- inine	Ergot- amine/- inine	Ergo- cornine/- inine	α -Ergo- cryptine/- inine	Ergo- cristine/- inine	Σ Ergot alkaloids
Mean	2.4	14	<14	<26	<8	22	<76
Median	1.5	<12	<14	<26	<8	<18	<76
Minimum	<0.3	<12	<14	<26	<8	<18	<76
Maximum	9.0	53	16	<26	19	112	206
SD*	2.5	13	2	0	4	26	42
% samples >dl*	75	40	5	0	15	30	15

Table 12 shows the results of total moulds, specific genera of toxigenic storage moulds (*Penicillium*, *Aspergillus*), Mucorales and yeasts in farm-mixed feed for pigs. The mould growth was dominated by *Penicillium spp.*, which was detected in ten of twelve samples. *Aspergillus spp.* exceeded the detection limit in three samples. The genus *Penicillium* and *Aspergillus* may produce a wide range of various mycotoxins. However, with the exception of OTA, these mycotoxins were not examined in the present surveillance. Yeasts were found in all samples of the farm-mixed pig feed.

Though there are no official guidance values for fungi in farm-mixed feed the presence of moulds and yeasts are good indicators of challenging hygienic quality of feed production and storage. It is desirable that this type of feed has a very low occurrence of mould (<100 cfu/g), and also a low occurrence of yeast (<100,000 cfu/g). All samples exceeded 100 cfu/g of mould and seven samples (58 %) exceeded 100,000 cfu/g of yeasts, indicating reduced hygienic quality.

The farm-mixed feed samples were also analysed for *Salmonella* with negative results.

The analysis of mycotoxins in farm-mixed feed for pigs showed low and insignificant levels (Tables 13 and 14).

Table 12. Occurrence of total moulds, *Penicillium* spp., *Aspergillus* spp., *Mucorales* and yeasts (cfu/g) in farm-mixed feed for pigs (N = 12) sampled in Norway in 2022.

	Total mould	<i>Penicillium</i>	<i>Aspergillus</i>	<i>Mucorales</i>	Yeasts
Mean	29 100	17 800	150	6 800	3 093 600
Median	1 300	680	<50	<50	450 000
Minimum	100	<50	<50	<50	150
Maximum	280 000	160 000	1 000	77 000	15 000 000
SD*	79 600	45 700	306	22 100	4 726 900
% samples >dl*	100	83	25	50	100

* SD = Standard Deviation, >dl = above detection limits.

Table 13. Concentrations ($\mu\text{g}/\text{kg}$) of deoxynivalenol (DON), DON-3-glucoside (DON-3-G), sum of T-2 and HT-2 toxin, nivalenol (NIV), zearalenone (ZEA) fumonisin B1 and B2, and ochratoxin A (OTA) in farm-mixed feed for pigs (N = 12) sampled in Norway in 2022.

	DON	DON-3-G	T-2 + HT-2	NIV	ZEA	OTA
Mean	21	<8	<62	<13	<14	<24
Median	<17	<8	<62	<13	<14	<24
Minimum	<17	<8	<62	<13	<14	<24
Maximum	55	11	<62	<13	<14	<24
SD*	19	2	0	0	0	0
% samples >dl*	33	17	0	0	0	0
% samples >gv*	0		0		0	0

* SD = Standard Deviation, >dl = above detection limits, >gv = above guidance values.

Table 14. Concentrations of ergot toxins ($\mu\text{g}/\text{kg}$) consisting of ergotamine/ergotaminine, ergocornine/ergocorninine, alpha-ergocryptine/alpha-ergocryptinine, ergocristine/ergocristinine and sum ergot alkaloids in farm-mixed feed for pigs (N = 12) sampled in Norway in 2022.

	Ergo-novine/-inine	Ergo-sine/-inine	Ergot-amine/-inine	Ergo-cornine/-inine	α -Ergo-cryptine/-inine	Ergo-cristine/-inine	Σ Ergot alkaloids
Mean	1.6	<12	<14	<26	<8	<18	<76
Median	0.7	<12	<14	<26	<8	<18	<76
Minimum	<0.3	<12	<14	<26	<8	<18	<76
Maximum	5.9	<12	<14	<26	11	<18	<76
SD*	1.9	0	0	0	2	0	5
% samples >dl*	50	0	0	0	8	0	0

Conclusions

Feed materials

- **Oats:** In 2022, the concentration of DON was at the lowest since the annual surveillance started in 2002. However, the DON level was higher in the Midt region than in regions in South-Eastern Norway, probably related to greater humidity during the growing season in Midt region compared to a correspondingly drier season in South-East. T-2/HT-2 toxin were below the average concentrations in the last decade, but one sample was above the guidance level after discounting analytical uncertainty.
- **Barley:** Insignificant levels of mycotoxins were found. Ergot was found in some samples at very low levels. No significant correlation between ergot and ergot alkaloids was present.
- **Rye:** Ergot was detected in all rye samples, in several at very high levels. Also ergot alkaloids were significantly elevated in several samples. Correlation between ergot and ergot alkaloids was not statistically significant. As the samples were taken from the batches before the cleansing of ergot, the results were not representative for rye distributed at the market.
- **Mycotoxins in maize:** Aflatoxins were detected in one out of eight samples with concentrations of aflatoxin B1, B2 and G1 detected at 18.2, 1.80 and 0.10 µg/kg, respectively. The sample did not exceed the maximum limit of aflatoxin B1 (20 µg/kg). The sample that contained aflatoxins was maize gluten, while the other samples were whole maize. ZEA was found in most samples, all below the guidance levels, and trace concentration of OTA was found in a single sample.

Feed

- **Compound feed for pig:** DON was found in most samples, all at insignificant concentrations. Other trichothecenes, ZEA, fumonisins and OTA were not or barely detectable. Ergot alkaloids were found at low levels in some samples.
- **Farm-mixed feed for pigs:** Fungi in farm-mixed feed consisted mostly of storage moulds and yeasts. The dominating storage moulds were *Penicillium* spp., detectable in 83 % of the samples. Yeasts were found in all samples. The level of fungi indicate reduced hygienic quality in several samples. *Salmonella* was not found. The levels of mycotoxins in farm-mixed feed were even lower than in the samples of compound feed.

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Appendix

Appendix Table 1. Results on mycotoxins (all in µg/kg) in 45 individual samples of oats from different regions in 2022. DON=deoxynivalenol, 3-Ac-DON=3-acetyl-DON, 15-Ac-DON=15-acetyl-DON, DON-3-G=DON-3-glucoside, T-2=T-2 toxin, HT-2=HT-2 toxin, NIV=nivalenol, ZEA=zearalenone.

ID-nr.	DON	3-Ac-DON	15-Ac-DON	DON-3G	T-2	HT-2	NIV	ZEA	Ergo- novine	Ergo- sine	Ergot- amine	Ergo- cornine	α-Ergo- cryptine	Ergo- cristine
OATS Region Øst														
2022-21-55-2	49	<25	<46	<8	65	88	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-56-1	<17	<25	<46	<8	80	234	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-57-1	399	<25	<46	27	131	311	59	<14	<0.3	14	<14	<25	<8	<18
2022-21-63-2	<17	<25	<46	<8	71	144	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-64-2	20	<25	<46	<8	31	55	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-65-2	45	<25	<46	<8	369	622	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-66-2	43	<25	<46	<8	<25	45	<13	<14	<0.3	106	<14	82	281	<18
2022-21-67-2	<17	<25	<46	<8	<25	66	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-96-2	36	<25	<46	23	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-97-2	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-107-6	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-107-7	43	<25	<46	<8	73	128	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-107-8	30	<25	<46	<8	60	154	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-123-1	35	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-123-2	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18
OATS Region Stor-Oslo														
2022-21-52-2	176	<25	<46	28	39	72	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-53-2	22	<25	<46	<8	54	129	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-54-2	24	<25	<46	<8	67	199	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-88-2	22	<25	<46	<8	28	50	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-89-2	45	<25	<46	<8	<25	77	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-90-2	71	<25	<46	<8	<25	58	<13	<14	<0.3	<11	<14	<25	<8	<18

2022-21-91-1	<17	<25	<46	<8	81	165	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-92-2	32	<25	<46	<8	43	141	39	<14	<0.3	<11	<14	<25	<8	<18
2022-21-93-2	248	<25	<46	25	244	291	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-94-1	22	<25	<46	14	54	174	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-95-1	38	<25	<46	<8	56	134	33	<14	<0.3	<11	<14	<25	<8	<18
2022-21-98-2	53	<25	<46	<8	<25	<37	25	<14	<0.3	<11	<14	<25	<8	<18
2022-21-99-2	147	<25	<46	16	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-100-2	48	<25	<46	<8	<25	<37	26	<14	<0.3	<11	<14	<25	<8	<18
2022-21-101-2	223	<25	<46	19	42	137	45	<14	<0.3	<11	<14	<25	<8	<18
2022-21-122-2	26	<25	<46	<8	34	61	65	<14	<0.3	<11	<14	<25	<8	<18
2022-21-126-2	37	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-127-2	25	<25	<46	<8	<25	62	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-128-2	20	<25	<46	<8	56	155	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-129-2	59	<25	<46	<8	44	130	<13	<14	<0.3	12	<14	<25	<8	<18
2022-21-145-2	45	<25	<46	21	35	56	21	<14	<0.3	<11	<14	<25	<8	<18
OATS Region Midt														
2022-21-85-2	178	<25	<46	<8	<25	76	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-85-4	270	<25	<46	<8	59	121	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-134-1	31	<25	<46	13	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-141-2	90	<25	<46	17	<25	<37	50	<14	<0.3	<11	<14	<25	<8	<18
2022-21-141-3	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-143-1	258	41	<46	83	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-143-2	459	37	<46	121	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18
2022-21-143-3	270	45	<46	126	<25	<37	<13	<14	<0.3	24	<14	56	34	<18
OATS Region Sør og Vest														
2022-21-46-2	<17	<25	<46	<8	<25	57	<13	<14	<0.3	<11	<14	<25	<8	<18

Appendix Table 2. Results on mycotoxins ($\mu\text{g}/\text{kg}$) and *Claviceps purpurea* (mg/kg) in barley based on 47 individual samples from different regions in 2022. DON=deoxynivalenol, 3-Ac-DON=3-acetyl-DON, 15-Ac-DON=15-acetyl-DON, DON-3-G=DON-3-glucoside, T-2=T-2 toxin, HT-2=HT-2 toxin, NIV=nivalenol, ZEA=zearalenone.

ID-nr.	DON	3-Ac-DON	15-Ac-DON	DON-3G	T-2	HT-2	NIV	ZEA	Ergo- novine	Ergo- sine	Ergot- amine	Ergo- cornine	α -Ergo- cryptine	Ergo- cristine	<i>C. purpurea</i> sclerotia
BARLEY Region Øst															
2022-21-55-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-63-1	28	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-64-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-65-1	25	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	5.6
2022-21-66-1	117	<25	<46	39	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	1.4
2022-21-67-1	<17	<25	<46	<8	37	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	7.7
2022-21-96-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	48	<14	<25	<8	65	0.0
2022-21-97-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-107-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	4.2
2022-21-107-2	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	20	<14	<25	12	64	5.1
2022-21-107-3	<17	<25	<46	<8	<25	<37	<13	<14	0.6	34	<14	<25	<8	60	54.9
2022-21-107-4	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	15.1
2022-21-107-5	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
BARLEY Region Stor-Oslo															
2022-21-52-1	58	<25	<46	43	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-53-1	35	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-54-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	18.8
2022-21-88-1	37	<25	<46	17	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	6.7
2022-21-89-1	<17	<25	<46	<8	<25	<37	46	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-90-1	32	<25	<46	18	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.6
2022-21-91-2	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-92-1	28	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-93-1	217	<25	<46	93	<25	<37	31	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-98-1	34	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-99-1	48	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	32.0
2022-21-100-1	23	<25	<46	<8	<25	<37	30	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-101-1	52	<25	<46	29	<25	<37	22	<14	<0.3	<11	<14	<25	<8	<18	0.0

2022-21-122-1	25	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-126-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-127-1	18	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	5.9
2022-21-128-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-129-1	27	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-144-1	35	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-145-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
BARLEY Region Midt															
2022-21-82-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	12	<14	<25	<8	<18	0.0
2022-21-83-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	36	<14	51	29	25	28.5
2022-21-84-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-85-1	<17	<25	<46	<8	80	38	<13	<14	<0.3	24	<14	<25	<8	216	0.0
2022-21-85-3	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-87-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-133-1	51	<25	<46	20	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-138-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-138-2	22	<25	<46	25	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-141-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	1.7
BARLEY Region Sør og Vest															
2022-21-46-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	12	<14	<25	<8	22	16.8
2022-21-46-3	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-46-4	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0
2022-21-125-1	<17	<25	<46	<8	<25	<37	<13	<14	<0.3	<11	<14	<25	<8	<18	0.0

Appendix Table 3. Results on mycotoxins ($\mu\text{g}/\text{kg}$) and *Claviceps purpurea* (mg/kg) in rye based on 14 individual samples from different regions in 2022.

n.a.=not analysed.

ID-nr.	Ergonovine	Ergosine	Ergotamine	Ergocornine	α -Ergocryptine	Ergocristine	Σ Ergot alkaloids	<i>C. purpurea</i> sclerotia
2022-21-73-1	<0.3	<11	<14	<25	<8	<18	<76	158
2022-21-73-2	<0.3	50	196	<25	<8	<18	272	295
2022-21-73-3	10.0	46	<14	<25	204	729	1010	1850
2022-21-73-4	16,8	85	21	74	197	23	418	4960
2022-21-73-5	<0.3	<11	<14	<25	<8	<18	<76	26
2022-21-73-6	9.8	114	187	94	639	263	1307	7910
2022-21-73-7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	797
2022-21-73-8	21.6	233	553	117	467	307	1699	2409
2022-21-73-9	8.6	60	162	27	55	220	532	2550
2022-21-73-10	12.2	328	154	91	230	239	1054	3470
2022-21-73-11	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	3450
2022-21-73-12	18.6	107	308	184	272	74	963	2500
2022-21-122-3	226.9	1461	2555	1256	2242	5415	13156	7021
2022-21-126-3	20.5	11	37	<25	136	228	445	304

Appendix Table 4. Results on mycotoxins in individual samples of complete feed for pigs (20 samples) 2022. All concentrations in µg/kg. DON=deoxynivalenol, 3-Ac-DON=3-acetyl-DON, 15-Ac-DON=15-acetyl-DON, DON-3-G=DON-3-glucoside, T-2=T-2 toxin, HT-2=HT-2 toxin, NIV=niivalenol, ZEA=zearalenone OTA= ochratoxin A, Fum B1=fumonisin B1, FumB2=fumonisin B2.

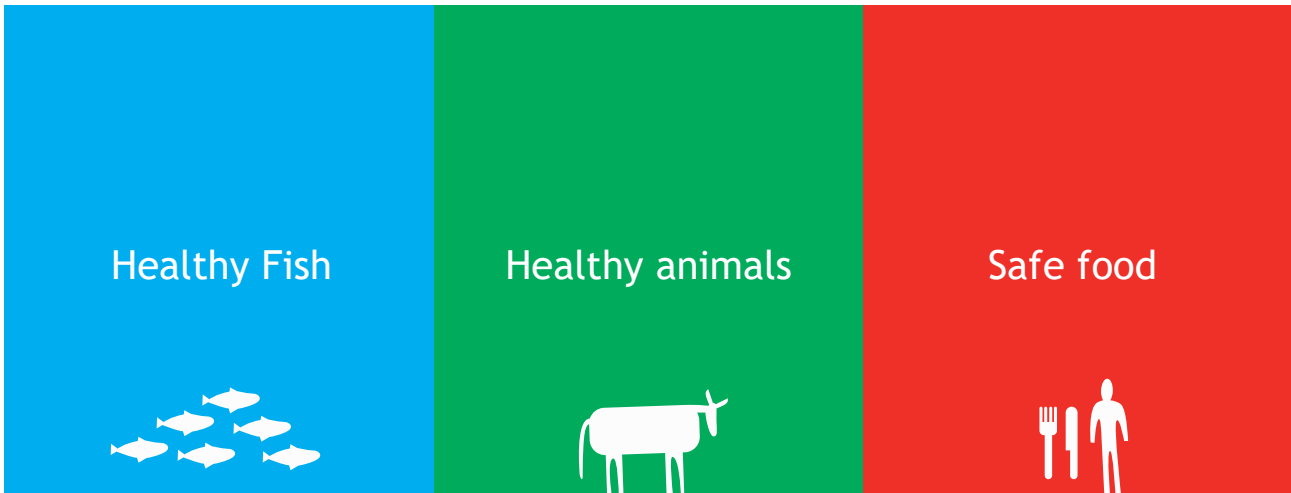
ID-nr.	Type of feed	DON	3-ac-DON	15-ac-DON	DON-3G	T-2	HT-2	NIV	ZEA	OTA	Fum B1	Fum B2	Ergo-novine	Ergo-sine	Ergot-amine	Ergo-cornine	α-ergo-cryptine	Ergo-cristine
2022-21-8-1	Opti Drekstig Våt	98	<25	<46	25	<25	<37	<13	<14	<24	<32	<14	<0.3	15	<14	<25	15	<18
2022-21-10-1	Opti Norm Komfort Våt	89	<25	<46	32	<25	<37	<13	<14	<24	<32	<14	4.0	<11	<14	<25	<8	<18
2022-21-11-1	Format Kvik 2	74	<25	<46	20	<25	<37	<13	<14	<24	<32	<14	<0.3	<11	<14	<25	<8	<18
2022-21-15-1	Format Soft 105	60	<25	<46	18	<25	<37	<13	<14	<24	<32	<14	6.4	<11	<14	<25	<8	<18
2022-21-16-1	Format Purke Soft	128	<25	<46	16	<25	<37	<13	<14	<24	<32	<14	4.1	<11	<14	<25	<8	<18
2022-21-19-1	Ideal 50	24	<25	<46	<8	<25	<37	<13	<14	<24	<32	<14	<0.3	<11	<14	<25	<8	<18
2022-21-32-1	Fullfôr til svin	70	<25	<46	<8	<25	<37	<13	<14	<24	<32	<14	0.5	26	<14	<25	11	23
2022-21-32-2	Fullfôr til svin	85	<25	<46	18	<25	<37	<13	<14	<24	<32	<14	5.0	28	<14	<25	<8	31
2022-21-35-1	Fullfôr til svin	44	<25	<46	<8	<25	<37	<13	<14	<24	<32	<14	0.5	<11	<14	<25	<8	<18
2022-21-36-1	Fullfôr til svin	29	<25	<46	<8	<25	<37	<13	<14	<24	<32	<14	<0.3	<11	<14	<25	<8	<18
2022-21-39-1	Fullfôr til svin	<17	<25	<46	<8	<25	<37	<13	<14	<24	<32	<14	2.8	21	<14	<25	<8	35
2022-21-48-1	Fullfôr til svin	<17	<25	<46	<8	<25	<37	<13	<14	<24	<32	<14	5.2	<11	<14	<25	<8	<18
2022-21-48-2	Fullfôr til svin	<17	<25	<46	<8	<25	<37	<13	<14	<24	<32	<14	1.9	53	<14	<25	19	112
2022-21-86-1	Fullfôr til svin	43	<25	<46	<8	<25	<37	<13	<14	<24	<32	<14	2.6	14	<14	<25	<8	<18
2022-21-110-1	Smågrisfor	19	<25	<46	<8	<25	<37	<13	16	<24	<32	<14	0.4	<11	<14	<25	<8	<18
2022-21-111-1	Ideal gjeldpurke	24	<25	<46	<8	<25	38	<13	<14	<24	<32	<14	1.0	33	<14	<25	<8	57
2022-21-112-1	Ideal 50 mysekombi	27	<25	<46	<8	<25	<37	<13	<14	<24	<32	<14	3.8	16	16	<25	<8	58
2022-21-142-1	Format Vekst 110	91	<25	<46	<8	<25	<37	<13	<14	<24	<32	<14	0.9	<11	<14	<25	<8	<18
2023-21-4-1	Format Komplet II	18	<25	<46	<8	<25	<37	<13	<14	<24	<32	16	9.0	<11	<14	<25	<8	<18
2023-21-5-1	Ideal junior	<17	<25	<46	<8	<25	<37	<13	<14	<24	62	16	<0.3	<11	<14	<25	<8	<18

Appendix Table 5. Results on fungi (total mould, *Penicillium* spp., *Aspergillus* spp., Mucorales, yeasts; all cfu/g) and mycotoxins (all µg/kg) in 12 individual samples of farm-mixed feed for pigs in 2022. DON=deoxynivalenol, 3-Ac-DON=3-acetyl-DON, 15-Ac-DON=15-acetyl-DON, DON-3-G=DON-3-glucoside, T-2=T-2 toxin, HT-2=HT-2 toxin, NIV=niivalenol, ZEA=zearalenone OTA= ochratoxin A.

ID-nr.	Salmonella	Total moulds	<i>Penicillium</i>	<i>Aspergillus</i>	Mucorales	Yeasts	DON	3-Ac-DON	15-Ac-DON	DON-3G	T-2+HT-2	NIV	ZEA	OTA	Σ Ergot alkaloids
2022-23-34-1	nd in 25 g	1200	860	<50	360	6500	41	<25	<46	8	<62	<13	<14	<24	<76
2022-23-35-1	nd in 25 g	280000	160000	<50	77000	1800000	<17	<25	<46	<8	<62	<13	<14	<24	<76
2022-23-38-1	nd in 25 g	31000	31000	<50	<50	7600000	<17	<25	<46	<8	<62	<13	<14	<24	<76
2022-23-42-1	nd in 25 g	10000	7700	<50	50	10000	<17	<25	<46	<8	<62	<13	<14	<24	<76
2022-23-59-1	nd in 25 g	1400	1400	<50	<50	670000	<17	<25	<46	<8	<62	<13	<14	<24	<76
2022-23-60-1	nd in 25 g	3500	500	<50	3000	4400000	<17	<25	<46	<8	<62	<13	<14	<24	<76
2022-23-61-1	nd in 25 g	100	50	50	<50	7400000	46	<25	<46	11	<62	<13	<14	<24	<76
2022-23-63-1	nd in 25 g	300	<50	<50	<50	230000	42	<25	<46	<8	<62	<13	<14	<24	<76
2022-23-73-1	nd in 25 g	800	100	550	50	650	<17	<25	<46	<8	<62	<13	<14	<24	<76
2022-23-74-1	nd in 25 g	150	<50	<50	<50	150	<17	<25	<46	<8	<62	<13	<14	<24	<76
2022-23-75-1	nd in 25 g	150	100	<50	<50	6200	55	<25	<46	<8	<62	<13	<14	<24	<76
2022-23-95-1	nd in 25 g	21000	12000	1000	1500	15000000	<17	<25	<46	<8	<62	<13	<14	<24	<76

Appendix Table 6. Results on aflatoxin B1, B2, G1, G2, ZEA=zearalenone, OTA=ochratoxin A ($\mu\text{g}/\text{kg}$) in 8 individual samples of maize 2022

ID-nr.	Type	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	ZEA	OTA
2022-21-12-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	<3.0	<0.1
2022-21-14-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	5.1	<0.1
2022-21-18-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	16.9	<0.1
2022-21-31-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	94.6	<0.1
2022-21-37-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	206	<0.1
2022-21-38-1	Maize gluten	18.2	1.80	0.10	<0.1	<3.0	0.3
2022-21-139-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	167	<0.1
2022-21-140-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	21.2	<0.1



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