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The surveillance programme for feed and feed materials in Norway 2021 – Mycotoxins and fungi

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Content

Summary

The surveillance programme for feed and feed materials in 2021 included selected fungi and mycotoxins in oats, barley, ryewheat (triticale) and in farm-mixed feed for pigs, selected mycotoxins in maize and in compound feed for pigs.

Concerning fungi in the cereal grains, oats showed total mould counts above the hygienic guidance value in 26 % of the samples, most consisting of field mould, which are mainly eliminated through normal drying process. In 7 % of the oat samples, *Fusarium* spp. were measured at levels considered of potential health concern when used as animal feed. Levels of total moulds and *Fusarium* were considered rather satisfactory compared to corresponding levels from the recent years. Levels of yeasts and storage moulds in oats were all within the guidance levels and considered at rather normal levels. In barley, total moulds above hygienic guidance value were found in 9 % of the samples. *Fusarium* spp. and storage moulds in barley were found in higher levels than in oats, with 17 % and 6 %, respectively above the guidance values. Yeasts were at a similar level as in oats. In barley was also determined *Claviceps purpurea*, which was detected in 49 % of the samples, all far below the maximum limit at 1000 mg/kg. In rye, *Claviceps purpurea* was detected in relatively more samples (79 %) than in barley but at a similarly low maximum level.

Concerning mycotoxins in cereal grains, T-2 and HT-2 toxin in oats were present at an average level compared with levels from recent years, with two samples above the guidance level after deduction of analytical uncertainty. Levels of deoxynivalenol (DON) and zearalenone (ZEA) in oats were low. In barley, mean levels of trichothecenes were only 1/5 to 1/3 of those in oats, whereas ZEA was at a similarly low level. Thus, the mycotoxin concentrations did not reflect the corresponding *Fusarium* levels. Ergot alkaloids in barley were present at lower concentrations than in most recent years. For the first time we observed in barley a significant correlation between the *Claviceps purpurea* and the ergot alkaloids. In ryewheat, the occurrence and concentrations of trichothecenes were even lower than in barley, whereas ZEA was a similar level. Ergot alkaloids were found at somewhat lower level in ryewheat than in barley.

Studies of regional differences of fungi and mycotoxins in oats and barley revealed in oats significantly more *Fusarium* and ZEA, and significantly less yeast and T-2/HT-2 in region Midt than in South-Eastern regions. In barley was revealed less yeast in region Midt than in South-Eastern regions, and more storage mould and less yeast in region Sør and Vest compared with the other regions.

In maize were found aflatoxins in two of 14 samples, below the maximum limit, and ZEA and ochratoxin A (OTA) in some samples below the guidance levels.

In compound feed for pigs were found the common trichothecenes in most samples, and ZEA and OTA in some samples, all below the guidance levels. In farm-mixed feed for pigs were found similar or lower levels of mycotoxins than in the compound feed samples. Determination of fungi revealed storage moulds, particularly *Penicillium* spp., and yeasts at high levels in several samples indicating reduced hygienic quality.

Sammendrag

Overvåkingsprogrammet for fôr og fôrmidler i 2021 omfattet utvalgte sopp og mykotoksiner i havre, bygg, rughvete og i gårdsblandet fôr til gris, og utvalgte mykotoksiner i mais og i fôrblandinger til gris.

Når det gjelder sopp i kornprøvene, viste havre totalt muggtall over hygienisk veiledende grense i 26 % av prøvene, det meste bestående av åkermugg, som hovedsakelig elimineres ved normal tørkeprosess. I 7 % av havreprøvene ble Fusarium spp. målt over et nivå som kan indikere potensiell fare for helseproblemer ved bruk som fôr. Nivåer av total muggsopp og Fusarium kan regnes som ganske tilfredsstillende sammenlignet med tilsvarende målinger fra de siste årene. Nivåer av gjær og lagringsmugg i havre var alle innenfor veiledende grenser og kan regnes som normale nivåer. I bygg ble det funnet totalt muggsopp over hygienisk veiledende grense i 9 % av prøvene. Fusarium spp. og lagringsmugg i bygg ble funnet i høyere nivåer enn i havre, med henholdsvis 17 % og 6 % over veiledende grenser. Gjærsopp var på samme nivå som i havre. I bygg ble det også bestemt Claviceps purpurea, som ble påvist i 49 % av prøvene, alle langt under maksimalgrensen på 1000 mg/kg. I rug ble Claviceps purpurea påvist i relativt flere prøver (79 %) enn i bygg, men på et tilsvarende lavt maksimumsnivå.

Når det gjelder mykotoksiner i korn, ble T-2 og HT-2 toksin i havre påvist på et normalt nivå sammenlignet med de siste årene, med to prøver over veiledende grense når analysemetodens usikkerhet er fratrukket. Nivåene av deoksynivalenol (DON) og zearalenon (ZEA) i havre var lave. I bygg var gjennomsnittlige nivåer av trichothecener bare 1/5 til 1/3 av de i havre, mens ZEA var på et tilsvarende lavt nivå. Mykotoksinkonsentrasjonene gjenspeilet derfor ikke Fusarium-nivåene. Ergot-alkaloider i bygg ble påvist i lavere konsentrasjoner i 2021 enn de siste årene. For første gang observerte vi i bygg en signifikant korrelasjon mellom Claviceps purpurea og ergotalkaloidene. I rughvete var forekomsten og konsentrasjonene av trichothecener enda lavere enn i bygg, mens ZEA hadde et tilsvarende nivå. Ergot-alkaloider ble funnet i noe lavere nivå i rughvete enn i bygg.

Undersøkelser av regionale forskjeller av sopp og mykotoksiner i havre og bygg viste signifikant mer *Fusarium* og ZEA i havre, og signifikant mindre gjær og T-2/HT-2 i region Midt enn i regioner i sørøst-Norge. I bygg ble det funnet mindre gjær i region Midt enn i regioner i sørøst, og mer lagringsmugg og mindre gjær i region Sør og Vest sammenlignet med de andre regionene.

I mais ble det funnet aflatoksiner i to av 14 prøver, begge under maksimumsgrensen, og ZEA og ochratoksin A (OTA) i noen prøver under veiledende grenser.

I kraftfôr til gris ble de vanlige trichothecenene funnet i de fleste prøvene, og ZEA og OTA i noen prøver, alle under veiledende grenser.I gårdsblandet fôr til gris ble det funnet tilsvarende eller lavere nivåer av mykotoksiner sammenlignet med kraftfôrprøvene. Ved undersøkelse av sopp ble det påvist lagringssopp, spesielt *Penicillium* spp., og gjær i høye nivåer i en del prøver, noe som indikerer redusert hygienisk kvalitet.

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 *Fusarium, Alternaria, Microdocchium, Cladosporium, Acremonium, Epicoccum, Phoma* and more. In addition, *Claviceps purpurea* (ergot), belong to field fungi [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 constitute variable amounts of 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 may 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. 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. Barley seems to be more susceptible to *C. purpurea* and their toxins 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 livestock effect of OTA is nephrotoxicity in pigs, but it may also suppress the immune response and growth performance [1]. As far as we know, OTA has not caused problems for Norwegian husbandry, but active surveillance of OTA is important, particularly because of imported feed ingredients [1].

In addition, aflatoxins produced by some Aspergilli, 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. 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 in Norway are to provide reliable documentation 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 2021, the surveillance programme for feed consisted of the following samples shown in Table 1.

Table 1*. Samples in the surveillance programme for feed 2021.*

Matrix	Planned	Sampled and analysed	Analyses
Oats	45	42	Total moulds, *Fusarium*, storage moulds, yeast, trichothecenes and zearalenone.
Barley	45	47	Total moulds, *Fusarium*, storage moulds, yeast, *Claviceps purpurea*, trichothecenes, zearalenone and ergot alkaloids.
Ryewheat (triticale)	20	14	*Claviceps purpurea*, trichothecenes, zearalenone and ergot alkaloids.
Maize/maize products	15	14	Aflatoxins, ochratoxin A and zearalenone.
Complete compound feed for pigs	20	20	Trichothecenes, zearalenone, ochratoxin A.
Farm-mixed feed for pigs	25	18	Total moulds, *Penicillium*, *Aspergillus*, Mucorales, yeast, trichothecenes, zearalenone and ochratoxin A.

Oats, barley and rye from mills in grain production areas were sampled during autumn. Maize from imported batches 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 691/2013.

Quantitative determination of fungi in oats and barley and farm-mixed feed for pigs

Quantitative determinations of total moulds and yeasts in oats and barley were performed by using NMKL method No 98 and using Malt-yeast-extract-sucrose-agar (MYSA) as growth medium. In addition, a qualitative determination of the composition of the mycoflora was performed by counting *Fusarium* and storage moulds separately. The detection limit was 50 colony-forming units per gram (cfu/g).

The most dominant *Fusarium* species in each sample were identified morphologically (Internal laboratory method ME02_151).

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 counting *Penicillium* and *Aspergillus* separately. The detection limit was 50 colony-forming units per gram (cfu/g).

Quantitative determination of *Claviceps purpurea* in barley and ryewheat

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.

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

In 2021 all samples were sent to Premier Analytical Services (PAS), England for mycotoxin analyses. The laboratories are 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 accredits 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.

All 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.

Trichothecenes were determined by gas chromatography with mass spectrometry (GC/MS). PAS uses the UKAS accredited method BA-TM-03 for the analysis of trichothecene mycotoxins. The test included the following mycotoxins: Deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), nivalenol (NIV), T2 toxin (T-2), HT-2 toxin (HT-2), fusarenone X (FUSX), diacetoxyscirpenol (DAS), neosolaniol (NEO). Limit of Quantification: 10 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.

Ergot alkaloids were determined by LC-MS/MS. PAS uses the UKAS accredited method BA-TM-33 for the analysis of ergot alkaloids. The test included the following compounds: Ergometrine, ergosine, ergocornine, ergotamine, ergocryptine, ergocristine, all with corresponding *–inine* forms. Limit of Quantification: 2 µg/kg for each toxin.
Aflatoxin: 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.

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.

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 specific to a variable were used for calculation purposes when levels were not detectable.

Results and discussion

Cereals

Fungi and mycotoxins in oats

In oats in 2021, total moulds, *Fusarium* spp., storage moulds, and yeasts were measured. Total mould counts, detectable in all samples, were above guidance value (500,000 cfu/g) [9] in 26 % of the samples (Table 2). The mean level of total moulds in oats 2021 varied little from those in the last years, but the number of samples above guidance level was considerably lower than in 2018-20 (46-64 %) [3-5]. Total moulds above guidance levels indicate poor hygienic quality if used directly. In general, feed of poor hygienic quality can cause reduced growth rate and create health problems in animals [10]. However, fresh grains from the field may naturally contain high levels of field fungi, which are eliminated through common drying process before usage as animal feed.

Fusarium spp., detected in all oat samples, had levels above 25,000 cfu/g in 7 % of the samples, which due to mycotoxin production, could be potentially hazardous if used as feed [9]. The *Fusarium* level for oats in 2021 is considered relatively low. The mean level was half and number of samples above 25,000 cfu/g was only 1/5 compared with results from 2020 [3].

Storage moulds were found in 76 % of the oat samples, but none exceeded the guidance value for storage moulds at 100,000 cfu/g [9]. The level may be considered as relatively "normal".

Yeasts were detected in all samples, but none exceeded the guidance value for yeasts at 10,000,000 cfu/g [9]. The levels of yeasts in 2021 for oats were somewhat higher than those reported the last years 2018-20 [3-5].

***Table 2**. Occurrence of fungi (cfu/g of total Fusarium spp., storage moulds and yeasts) and mycotoxins (µg/kg of trichothecenes (deoxynivalenol (DON), 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON), T-2 and HT-2 toxin and the sum of T-2/HT-2, nivalenol (NIV)) and zearalenone (ZEA)) in **oats** (N = 42) sampled in Norway in 2021.*

	Total mould	*Fusarium* spp.	Storage mould	Yeasts	DON	3-Ac-DON	15-Ac-DON	T-2	HT-2	T-2+ HT-2	NIV	ZEA
Mean	614 000	12 700	4 500	1 234 000	229	20	<10	61	137	198	64	7
Median	410 000	5 000	250	1 000 000	113	<10	<10	46	105	148	49	<3
Minimum	55 000	500	<50	27 000	31	<10	<10	<10	<10	<20	<10	<3
Maximum	4 300 000	100 000	73 000	7 200 000	1190	181	<10	334	659	993	365	101
SD*	805 000	21 900	12 300	1 220 000	269	33	0	67	140	204	68	19
% samples >dl*	100	100	76	100	100	43	0	90	93	90	95	10
% samples >gv*	26	7	0	0	0					7		0

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

In 2021, rather low correlations were found between the levels of various groups of fungi in the oat samples. Only total moulds and yeasts were significantly correlated (Table 3). This result is in contrast to findings from most previous years, where more correlations between groups of fungi, and particularly a positive correlation between total moulds and *Fusarium* were often found [3-5].

Table 3. *Correlation coefficients between counts of the various groups of fungi in* ***oats*** *(N = 42) sampled in Norway in 2021. Counts significantly correlated (p<0.05) are given given in bold.*

	Total mould	*Fusarium* spp.	Storage mould	Yeasts
Total mould	1.000			
Fusarium spp.	0.249	1.000		
Storage mould	-0.036	-0.066	1.000	
Yeasts	**0.460**	-0.112	0.192	1.000

The frequencies of the dominating *Fusarium* species found in oats in 2021 are presented in Table 4. The order is the same as in 2020 [3], with only difference that number of samples with detected *Fusarium* species were lower in 2021. The dominance of *Fusarium poae* is also a common finding from all last years, as well as a certain occurrence of *F. langsethiae* and *F. graminearum* [3-5]. *F. poae* may produce NIV and ZEA and other secondary fungal metabolites but not DON [11]. *F. langsethiae* and *F. graminearum* are the main producers of T-2/HT-2 and DON, respectively [1]. *F. avenaceum* does not produce trichothecenes, but produces mycotoxins considered of less importance, such as moniliformin, enniatins and others [1]. Occurrence of the *Fusarium* species does not necessary mean occurrence of toxins as stressors for toxin production may not be present or the cereal plant has implemented toxin preventive mechanisms [12].

Table 4. *The frequency of the dominating Fusarium species found in* ***oats*** *(N = 42) sampled in Norway in 2021.*

Species	Number (%) of samples detected	Number of samples with most dominant species
F. poae	21 (50%)	18
F. avenaceum	12 (29%)	6
F. langsethiae	8 (19%)	7
F. graminearum	6 (14%)	3
F. tricinctum	5 (12%)	2
F. culmorum	1 (2%)	1

In 2021, DON was detected in all samples (Table 2), but at similarly low concentrations as in 2017-2020 [3-6]. All samples had levels far below the limit for DON recommended by EU and Norway (8000 µg/kg) [9, 13].

The DON-related compounds included in the analysis of oats were the acetylated precursor compounds (3-Ac-DON and 15-Ac-DON). 3-Ac-DON was found in 43 % of the samples, whereas 15-Ac-DON was not detected. DON and 3-Ac-DON were significantly positively correlated (Figure 1), which is also observed previous years [3-5]. In average, 3-Ac-DON was present at about 10 % level compared to DON, which is similar with the average in European cereals [14].

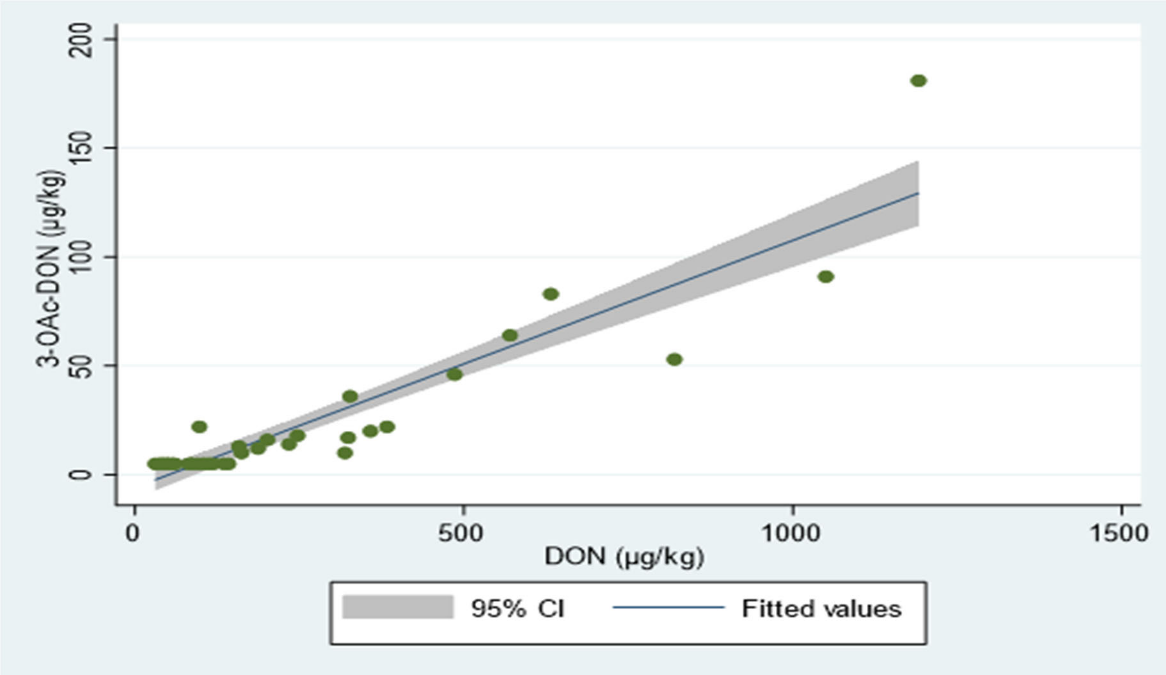

Figure 1. *The Pearson correlation between deoxynivalenol (DON) and 3-acetyl-DON in* ***oats*** *(N=42) 2021 was r=0.92, p<0.0001. A regression line with 95 % confidence interval fitted to the points allows predictions of levels of 3-OAc-DON given the level of DON detected and vice versa.*

T-2 and HT-2 were present in most oat samples. Three samples (7 %) showed the combined sum T-2+HT-2 concentrations numerically above the guidance level of 500 µg/kg in EU and Norway [9, 15] (Table 2; Figure 2). However, one of the samples had not regulatory exceedance of the limit when included the uncertainty of the analytical method. Thus, two samples exceed the guidance level after subtraction of the uncertainty. The mean concentration of T-2+HT-2 was not very different from most recent years but somewhat lower than in 2019 [3-7]. T-2 and HT-2 were highly correlated in oats, with an average concentration of HT-2 twice that of T-2 (Figure 2), which is similar with previous years [3-7].

The analytical method for trichothecenes revealed trace concentrations of neosolaniol in some samples: in 13 oat samples between 10 and 37 µg/kg, and in five barley samples between 10 and 15 µg/kg.

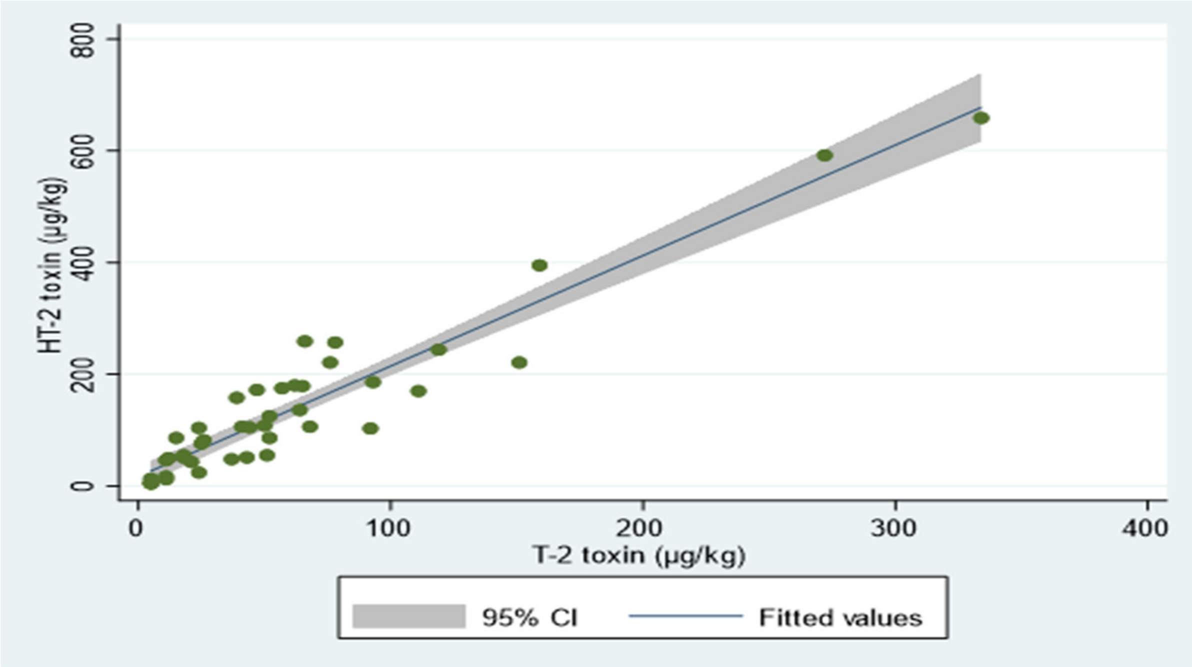

*Figure 2. The Pearson correlation between T-2 toxin and HT-2 toxin in **oats** (N=42) 2021 was r=0.95, p<0.0001. A regression line with 95 % confidence interval fitted to the points allows predictions of levels of HT-2 toxin given the level of T-2 toxin detected and vice versa.*

The concentrations of DON and T-2 + HT-2 in oats have been determined in surveillance programs in 20 years, since 2002 (Figures 3 and 4). The mean DON concentration in 2021 was similar to the last previous years – at the lower end of the scale (illustrated in Figure 3). In fact, the DON concentrations have been low since the peak DON levels in 2012. Furthermore, Figure 4 shows that the mean concentration of T-2+HT-2 in 2020 was at a rather average level.

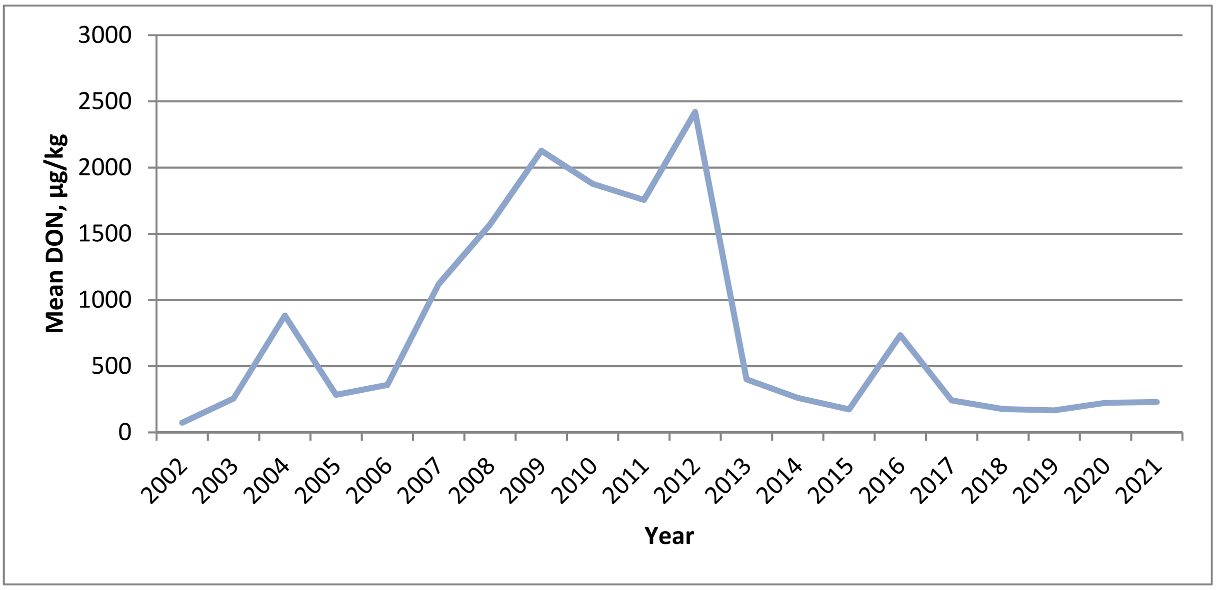

*Figure 3. Mean concentration of deoxynivalenol (DON) in 30-60 samples of **oats** per year in the Norwegian surveillance programme for feed.*

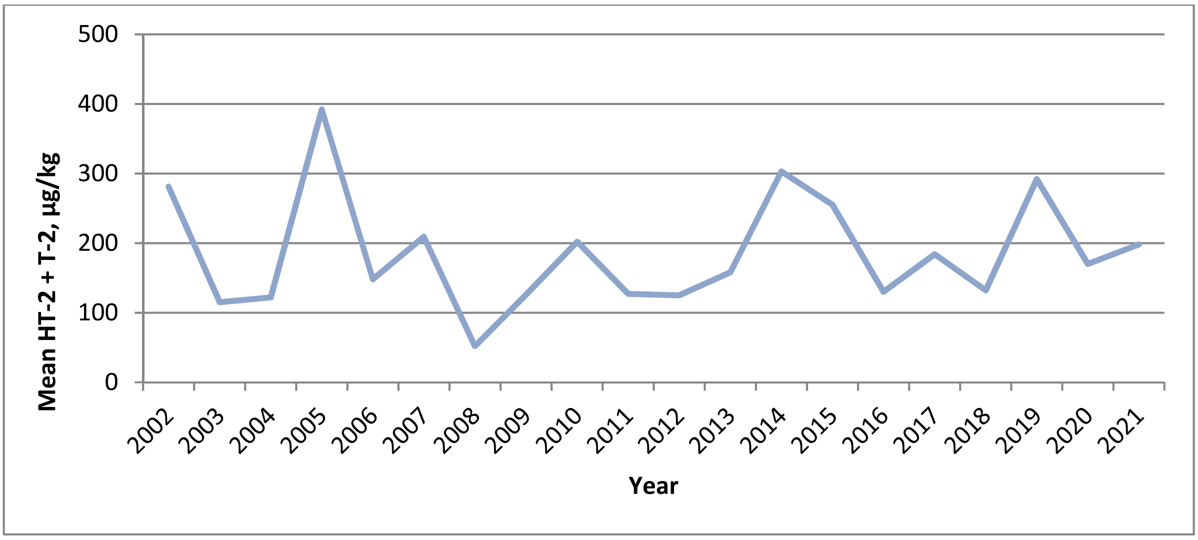

Figure 4*. Mean concentration of the sum of T-2 toxin and HT-2 toxin in 30-60 samples of* ***oats*** *per year in the Norwegian surveillance programme for feed.*

NIV was detected at low level (Table 2) with similar mean level as in 2020 [3]. The much lower occurrence of NIV than DON is as usual in Norway.

ZEA, detected in a few samples, were in trace amounts, similar as in 2020 [3], and far below the recommended limit (2000 µg/kg) in EU and Norway (Table 2) [9, 13].

The weather during the growing season is a key factor for the *Fusarium* and mycotoxin contents of cereal grains. In addition to the temperature is the level of precipitation and humidity during flowering (usually in July), as well as precipitation up to harvest in autumn, of particular importance [1]. July 2021 in South-Eastern and Midt regions was relatively warm with somewhat less precipitation than normally [16]. August in South-Eastern regions was averagely warm with relatively little precipitation, and September relatively warm with locally variable precipitation. In region Midt had August lower temperature and averagely more precipitation than normally, and September average temperature and precipitation. The weather for cereal production in these regions must be considered as relatively good. The results on moulds and mycotoxins in 2021 reflects good weather conditions.

The pattern of fungi and mycotoxins in 2021 showed rather satisfactory levels of total moulds, low levels of *Fusarium*, normal storage moulds, relatively high levels of yeasts, low concentrations of DON and average concentrations of T-2/HT-2 compared with previous years [3-7].

Fungi and mycotoxins in oats were analysed for regional differences (Table 5). Significantly more *Fusarium* were found in region Midt (Trøndelag, Møre, Romsdal) than in South-Eastern regions (Øst and Stor-Oslo). Concerning yeasts, significantly higher levels were found in South-Eastern regions that in region Midt. For mycotoxins, significantly higher levels of T-2/HT-2 were found in region Øst than in Midt. The lower of T-2/HT-2 in Trøndelag than in South-Eastern Norway is a common finding [4]. In contrast, the levels of ZEA was significantly higher in Midt than South-East.

***Table 5**. Survey between regions Øst (Buskerud, Vestfold, Telemark, Innlandet), Stor-Oslo (Akershus, Oslo, Østfold) and Midt (Trøndelag, Møre, Romsdal) on fungi (total moulds, Fusarium spp., storage moulds and yeast; all fungi cfu/g) and trichothecenes (deoxynivalenol (DON), sum of T-2 and HT-2 toxin, nivalenol (NIV)) and zearalenone (ZEA); all toxin concentrations µg/kg) in **oats** (N = 42) sampled in Norway in 2021. Variables that were significantly different between regions are indicated by an * (p<0.05).*

Region		Total mould	*Fusarium* spp.*	Storage mould	Yeast*	DON	T-2+ HT-2*	NIV	ZEA*
Øst n=15	Mean	847 000	9 300	1 800	1 131 000	213	296	61	<3
	St. dev.	1 242 000	13 600	4 400	804 000	306	229	31	2
Stor-Oslo n=19	Mean	527 000	7 400	6 200	1 712 000	161	164	58	<3
	St. dev.	368 000	5 800	17 300	1 480 000	128	197	80	3
Midt n=8	Mean	382 000	32 100	5 800	295 000	422	98	82	25
	St. dev.	399 000	42 500	6 900	377 000	372	60	90	40

Fungi and mycotoxins in barley

Total mould counts were found at lower levels in barley than in oats, which are also observed previously (Table 6) [5]. In contrast in 2021, *Fusarium* and storage moulds were found at higher levels in barley than in oats, which are not a static phenomenon and may vary year by year [5]. The levels of yeasts were similar between cereal species.

In barley total moulds were significantly correlated to *Fusarium*, in particular, and also to storage moulds and yeasts (Table 7). Whereas correlation between total moulds and *Fusarium* is a common finding, other correlations may vary year by year [3-5].

What is fixed year by year, is the lower concentrations of trichothecenes in barley compared with oats. Table 6 shows the 2021 concentrations of the trichthecenes DON, T-2, HT-2 and NIV as well as that of ZEA in barley. Mean levels of trichothecenes were 1/5 to 1/3 of those in oats. In contrast, ZEA showed similar levels in the two species.

Claviceps purpurea (ergot) and ergot alkaloids were also determined in barley (Table 8) as they are usually more present in this species than in oats. *C. purpurea* was detected in 49 % of the samples. The ergot had an overall mean level of 7 mg/kg and a maximum of 68 mg/kg. Thus, all samples were far below the legislated maximum concentration of 1000 mg/kg [9]. The occurrence of ergot was at a similar level as observed in barley in 2019 and 2020 [3-4]. Much of the ergot sclerotia were small in size, indicating they had been growing on grass. Only five samples (11 % of the samples) had sclerotia of the same size or bigger than the barley grain/kernel, indicating that the ergot had grown on the barley.

The ergot alkaloids were present at rather low levels in barley with a maximum concentration of sum alkaloids 547 µg/kg in 2021. The alkaloids were detectable in 32 % of the samples, which is higher than previously, due to a more sensitive analytical method.
Ergot alkaloids have been included in the analysis repertoire of barley since 2016. They have been only sporadically present. 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 for these batches were used as major feed ingredients

[6]. In 2020, a maximum of 1010 µg/kg was shown [3]. Knowledge on possible influence of climate or weather conditions on occurrence of ergot and ergot alkaloids is lacking.

Table 6. *Occurrence of fungi (cfu/g of total Fusarium spp., storage moulds and yeasts) and mycotoxins (µg/kg of trichothecenes (deoxynivalenol (DON), 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON), T-2 and HT-2 toxin and the sum of T-2/HT-2, nivalenol (NIV)) and zearalenone (ZEA)) in* ***barley*** *(N = 47) sampled in Norway in 2021.*

	Total mould	*Fusarium* spp.	Storage mould	Yeast	DON	3-Ac-DON	15-Ac-DON	T-2	HT-2	T-2+ HT-2	NIV	ZEA
Mean	251 000	17 900	27 900	1 352 000	94	<10	<10	15	27	42	21	6
Median	170 000	5 000	500	910 000	56	<10	<10	<10	11	<20	11	<3
Minimum	13 000	500	<50	41 000	<10	<10	<10	<10	<10	<20	<10	<3
Maximum	1 300 000	150 000	550 000	6 200 000	431	25	<10	109	288	397	156	105
SD*	246 000	32 500	95 500	1 175 000	94	4	0	24	46	67	28	17
% samples >dl*	100	100	76	100	98	17	0	30	53	47	55	21
% samples >gv*	9	17	6	0	0					0		0

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

Table 7. *Correlation coefficients between counts of the various groups of fungi in barley (N = 47) sampled in Norway in 2021. Counts significantly correlated (p<0.05) are given given in bold.*

	Total mould	*Fusarium* spp.	Storage mould	Yeast
Total moulds	1.000			
Fusarium spp.	**0.611**	1.000		
Storage moulds	**0.349**	0.002	1.000	
Yeasts	**0.407**	0.017	-0.241	1.000

Table 8. *Occurrence of Claviceps purpurea sclerotia (mg/kg) and ergot toxins (µg/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 2021.*

	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	7	3	15	<2	11	5	10	46
Median	0	<2	<2	<2	<2	<2	<2	<12
Minimum	0	<2	<2	<2	<2	<2	<2	<12
Maximum	68	86	164	32	328	102	111	547
SD*	15	12	33	5	49	16	23	96
% samples >dl*	49	11	26	6	11	19	28	32

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

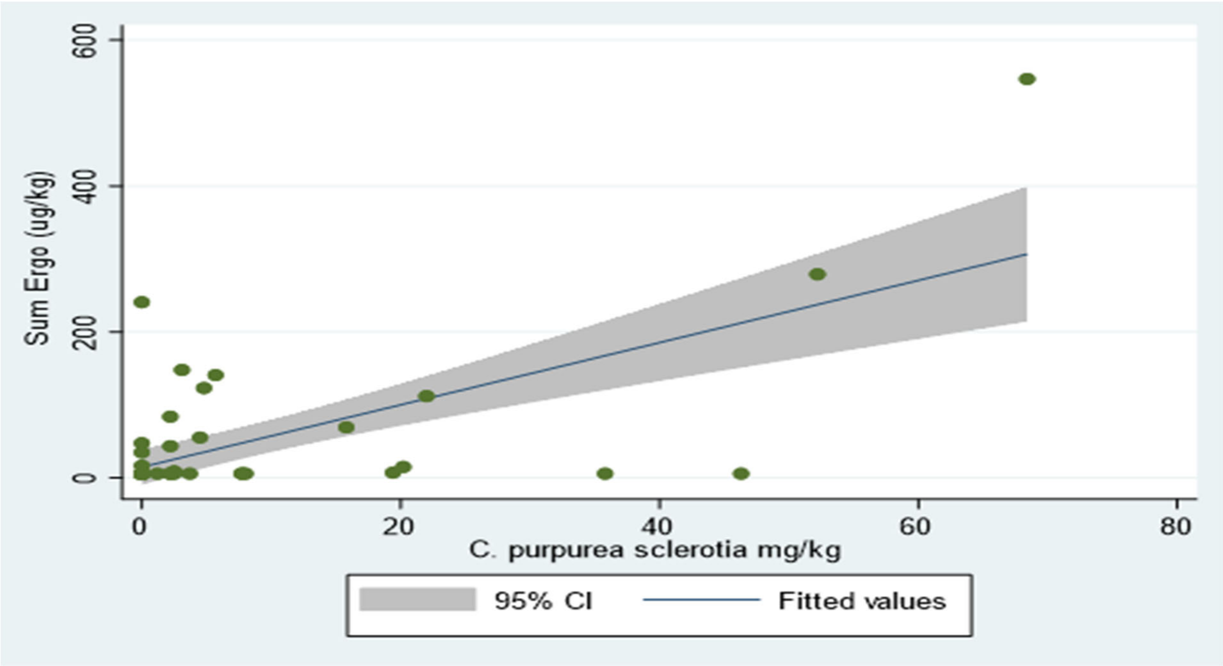

Figure 5*. The Pearson correlation between* *Claviceps purpurea* *and sum of ergot alkaloids in samples of* ***barley*** *(n = 47) 2021 was r=0.66, p<0.0001. A regression line fitted to the points with 95 % confidence interval is shown.*

For the first time we observed a significant correlation between the ergot and the ergot alkaloids (Figure 5). 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, 17-18]. The low or lack of correlation indicate variable production of alkaloids by the ergot fungi.

Fungi and mycotoxins in barley were analysed for regional differences (Table 9). The only significant differences were more storage mould and less yeast in region Sør and Vest compared with the other regions, as well as less yeast in region Midt than in South-Eastern regions (Øst and Stor-Oslo). Otherwise, the pattern showing more *Fusarium*, less T-2/HT-2 and more ZEA in region Midt than in South-Eastern regions were similar in barley and oats, but these trends were not statically significant in barley.

***Table 9**. Survey between regions Sør/Vest (Agder, Rogaland, Vestland), Øst (Buskerud, Vestfold, Telemark, Innlandet), Stor-Oslo (Akershus, Oslo, Østfold) and Midt (Trøndelag, Møre, Romsdal) on fungi (total moulds, Fusarium spp., storage moulds and yeast; all cfu/g), Claviceps purpurea (mg/kg) and trichothecenes (deoxynivalenol (DON), sum of T-2 and HT-2 toxin, nivalenol (NIV)), zearalenone (ZEA) and sum of ergot alkaloider; all toxin concentrations µg/kg) in **barley** (N = 47) sampled in Norway in 2021. Variables that were significantly different between regions are indicated by an * (p<0.05).*

Region		Total mould	*Fusarium* spp.	Storage mould*	Yeast*	DON	T-2+ HT-2	NIV	ZEA	*C.pur.*	Ergot alk.
Sør og Vest n=5	Mean	304 000	6 100	188 400	206 000	52	<20	11	<3	6	35
	St. dev.	254 000	2 400	249 200	174 000	32	0	7	1	8	63
Øst n=13	Mean	178 000	6 700	13 900	1 356 000	86	52	20	<3	11	54
	St. dev.	94 000	6 600	36 600	795 000	70	56	21	4	16	78
Stor-Oslo n=19	Mean	311 000	21 300	4 000	1 988 000	100	57	30	3	8	46
	St. dev.	316 000	33 300	8 700	1 391 000	92	91	38	7	18	125
Midt n=10	Mean	207 000	32 000	11 100	713 000	115	<20	10	18	2	40
	St. dev.	224 000	51 200	31 300	547 000	139	12	12	33	7	78

Fungi and mycotoxins in ryewheat (triticale)
In ryewheat were determined the ergot (*Claviceps purpurea*), ergot alkaloids, trichothecenes and zearalenone.

Claviceps purpurea was detected in relatively more samples of ryewheat than of barley, 79 vs. 49 %, respectively, and with a somewhat higher mean and maximum levels (Table 10). However, all samples were far below legislated maximum ergot level. Ergot alkaloids were detectable in similar percentages in ryewheat and barley, but with a somewhat lower mean and maximum levels in ryewheat than in barley (Tables 8 and 10). No correlation between ergot and ergot alkaloids was revealed (Figure 6).

The occurrence and concentrations of trichothecenes and ZEA in ryewheat were even lower than in barley (Table 11). Too few samples of ryewheat were collected to include studies of regional variations.

***Table 10**. Occurrence of Claviceps purpurea sclerotia (mg/kg) and ergot toxins (µg/kg) consisting of ergotamine/ergotaminine, ergocornine/ergocorninine, alpha-ergocryptine/alpha-ergocryptinine, ergocristine/ergocristinine and sum ergot alkaloids in **ryewheat** (N = 14) sampled in Norway in 2021.*

	***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	19	2	6	<2	<2	<2	6	19
Median	12	<2	<2	<2	<2	<2	<2	<12
Minimum	0	<2	<2	<2	<2	<2	<2	<12
Maximum	96	10	36	12	12	4	56	93
SD*	25	3	9	3	3	1	15	26
% samples >dl*	79	21	36	7	14	14	14	29

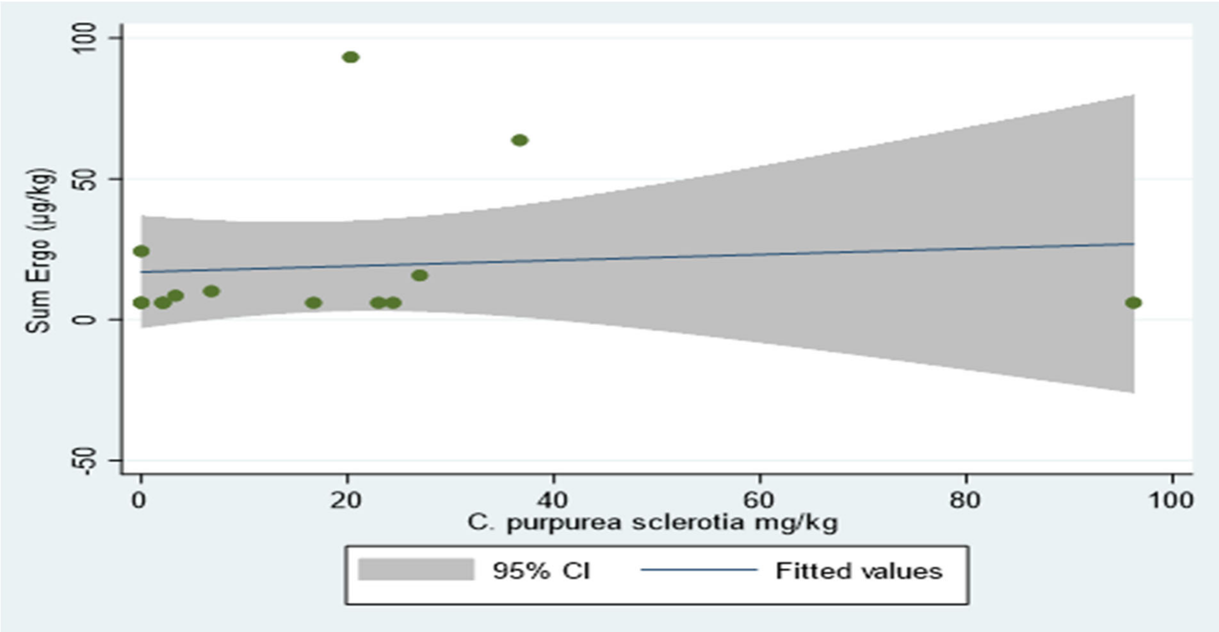

*Figure 6. The Pearson correlation between Claviceps purpurea and sum of ergot alkaloids in samples of **ryewheat** (n = 14) 2021 was r=0.10, p=0.74. A regression line fitted to the points with 95 % confidence interval is shown.*

*Table 11. Occurrence of trichothecene mycotoxins (deoxynivalenol (DON), 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON), T-2 and HT-2 toxin and the sum of T-2/HT-2, nivalenol (NIV)) and zearalenone (ZEA)) (all µg/kg) in **ryewheat** (N = 14) sampled in Norway in 2021.*

	DON	3-Ac-DON	15-Ac-DON	T-2	HT-2	T-2+ HT-2	NIV	ZEA
Mean	67	<10	<10	<10	<10	<20	<10	<3
Median	36	<10	<10	<10	<10	<20	<10	<3
Minimum	<10	<10	<10	<10	<10	<20	<10	<3
Maximum	306	<10	<10	15	16	31	24	5
SD*	79	0	0	4	4	7	6	1
% samples >dl*	86	0	0	29	50	29	36	7
% samples >gv*	0					0		0

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

Aflatoxins in maize

Aflatoxins were detectable in two of 14 analysed maize samples, with maximum concentrations of aflatoxin B1, B2 and G1 detected at 4.9, 0.30 and 0.20 µg/kg, respectively (Table 12). Thus, no samples exceed the maximum limit of aflatoxin B1 (20 µg/kg) [19].

In 2021, also OTA and ZEA were analysed in maize (Table 12). Trace concentrations of OTA were detectable in two maize samples far below the guidance level of 250 µg/kg [9]. ZEA were detectable in five samples up to 419 µg/kg, which is below guidance level of 3000 µg/kg [9].

*Table 12. Concentrations (µg/kg) of aflatoxins (B1, B2, G1, G2), ochratoxin A (OTA) and zearalenone (ZEA) in **maize** (N = 14) sampled in Norway in 2021.*

	Afla B1	Afla B2	Afla G1	Afla G2	OTA	ZEA
Mean	0.58	<0.10	<0.10	<0.10	<0.10	61
Median	<0.10	<0.10	<0.10	<0.10	<0.10	<3
Minimum	<0.10	<0.10	<0.10	<0.10	<0.10	<3
Maximum	4.9	0.30	0.20	<0.10	0.20	419
SD*	1.4	0.07	0.04	0	0.04	116
% samples >dl*	14	14	7	0	14	36
% samples >gv*	0				0	0

Feed

Feed for pigs

In 2021, complete compound feed for pigs were analysed for trichothecenes, ZEA and OTA, and farm-mixed feed for pigs were analysed for these mycotoxins as well as various groups of fungi.

The results on mycotoxins in complete compound feed for pigs in Table 13 show that DON was detected in all 20 samples. All were below the guidance level of DON for pig feed in Norway (500 µg/kg) [9]. Co-occurrence of DON-related compounds were mostly undetectable, with only trace amounts of 3-Ac-DON in three samples. Related compounds of DON can be an additional factor to the total DON exposure and EFSA considers their toxic effects like that of DON [14].

Also the sum of T-2 and HT-2 was present in most samples of the compound feed for pigs without exceeding the guidance level (250 µg/kg) [9, 15]. NIV was present in most samples but at insignificant levels.

ZEA was present at low concentrations in four samples of the compound feed, all below the guidance level for pig feed in Norway (250 µg/kg) [9].

OTA was detectable in 11 samples of the compound feed – all far below the guidance level (10 µg/kg in feed for pigs) [9].

***Table 13**. Concentrations (µg/kg) of deoxynivalenol (DON), 3-acetyl-DON, 15-acetyl-DON, T-2, HT-2, sum of T-2 and HT-2 toxin, nivalenol (NIV), zearalenone (ZEA) and ochratoxin A (OTA) in **complete compound feed for pigs** (N = 20) sampled in Norway in 2021.*

	DON	3-Ac-DON	15-Ac-DON	T-2	HT-2	T-2+ HT-2	NIV	ZEA	OTA
Mean	116	<10	<10	15	39	54	20	8	0.25
Median	99	<10	<10	13	34	43	16	<3	<0.10
Minimum	44	<10	<10	<10	<10	<20	<10	<3	<0.10
Maximum	270	15	<10	43	112	155	83	26	1.4
SD*	63	3	0	11	27	37	18	9	0.35
% samples >dl*	100	15	0	60	95	85	80	20	55
% samples >gv*	0					0		0	0

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

In farm-mixed feed for pigs, the mycotoxin levels were similar or lower than in the compound feed samples and none exceeded the guidance levels (Table 14).

The fungi analysed in farm-mixed feed for pigs consisted of total moulds, specific genera of toxigenic storage moulds (*Penicillium*, *Aspergillus*), Mucorales and yeasts (Table 14). Most of the mould growth consisted of storage moulds *Penicillium*, *Aspergillus* and Mucorales with dominance of *Penicillium* spp. which was detectable in 14 of the 18 samples. *Aspergillus* spp. exceeded the detection limit in nine samples. The genus *Penicillium* and *Aspergillus* may produce a wide range of various mycotoxins. However, such mycotoxins were not examined in the present programme.

***Table 14**. Occurrence of total moulds, Penicillium spp., Aspergillus spp., Mucorales, yeasts (cfu/g), and mycotoxins (µg/kg) of trichothecenes (deoxynivalenol (DON), 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON), sum of HT-2 and T-2 toxin, nivalenol (NIV)), zearalenone (ZEA) and ochratoxin A (OTA) in samples of **farm-mixed feed for pigs** (N = 18) sampled in Norway in 2021.*

	Total mould	*Penicil-lium*	*Asper-gillus*	Mucor-ales	Yeast	DON	3-Ac-DON	15-Ac-DON	T-2+ HT-2	NIV	ZEA	OTA
Mean	15 200	9 600	1000	500	5 352 000	32	<10	<10	<20	<10	<3	0.20
Median	800	300	<50	<50	905 000	19	<10	<10	<20	<10	<3	<0.10
Minimum	<50	<50	<50	<50	1 500	<10	<10	<10	<20	<10	<3	<0.10
Maximum	170 000	120 000	9 100	3 600	33 000 000	230	13	<10	20	12	9	2.0
SD*	40 200	29 200	2 400	1 200	8 977 000	51	2	0	2	2	2	0.46
% samples >dl*	89	78	50	22	100	89	6	0	6	6	22	44

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

Yeasts were found in all samples of the farm-mixed pig feed.

In farm-mixed feed for pigs there is no official guidance values. However, when it comes to ensuring good hygienic quality there are some challenges. 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). 15 samples (83 %) exceeded 100 cfu/g of mould and 11 samples (61 %) exceeded 100,000 cfu/g of yeast, indicating reduced hygienic quality. The sample with maximum yeasts of 33 million cfu/g also contained the maximum mould at 170,000 cfu/g.

Conclusions

Feed materials

- **Fungi in oats**: In 26 % of the samples, total mould counts were higher than the hygienic guidance value. Most consisted of field moulds which are mostly eliminated through normal drying process. In 7 % of the samples, *Fusarium* spp. were measured at levels considered of potential health concern (guidance value). The dominating *Fusarium* species were *F. poae*, *F. avenaceum and F. langsethiae*. Levels of total moulds and *Fusarium* were considered rather satisfactory compared to corresponding levels from the recent years. Levels of yeasts and storage moulds were all within the guidance levels and considered rather normal. Study of regional differences revealed significantly more *Fusarium* and significantly less yeast in region Midt than in South-Eastern regions.
- **Mycotoxins in oats:** Levels of DON and ZEA were low. Those of T-2 and HT-2 were at an average level compared with levels from recent years, with two samples above the guidance level after deduction of the analytical uncertainty. Significantly less T-2/HT-2 were present in region Midt than in region Øst, whereas the opposite was found for ZEA.
- **Fungi in barley:** Total moulds above hygienic guidance value were found in 9 % of the samples. *Fusarium* spp. and storage moulds in barley were found in higher levels than in oats, with 17 % and 6 %, respectively above the guidance values. Yeasts were at a similar level as in oats. *Claviceps purpurea*, detected in 49 % of the samples, were at levels far below the maximum limit at 1000 mg/kg. The significant regional differences were more storage mould and less yeast in region Sør and Vest compared with the other regions, as well as less yeast in region Midt than in South-Eastern regions.
- **Mycotoxins in barley:** Mean levels of trichothecenes were only 1/5 to 1/3 of those in oats, whereas ZEA was at a similarly low level. Ergot alkaloids were present at lower concentrations than in most recent years. No significant regional differences for mycotoxins were revealed.
- **Fungi in ryewheat:** *Claviceps purpurea* was detected in relatively more samples of ryewheat (79 %) than of barley but at a similarly low maximum level.
- **Mycotoxins in ryewheat:** The occurrence and concentrations of trichothecenes in ryewheat were even lower than in barley, whereas ZEA was at a similar level. Ergot alkaloids were found at somewhat lower level in ryewheat than in barley.
- **Mycotoxins in maize:** Aflatoxins were detectable in two out of fourteen samples. The highest concentration of aflatoxin B1 was 4.9 µg/kg and did not exceed the maximum limit (20 µg/kg). ZEA and OTA were found in some maize samples far below their respective guidance levels.

Feed

- **Compound feed for pig:** DON, T-2/HT-2 and NIV were present in most samples but all below guidance levels for feed for pigs. ZEA and OTA were found in some samples all below the guidance levels.

- **Farm-mixed feed for pigs:** The mycotoxin levels were similar or lower than in the compound feed samples and none exceeded the guidance levels. Fungi in farm-mixed feed consisted of storage moulds and yeasts. The dominating storage moulds were *Penicillium* spp., detectable in 78 % of the samples. Yeasts were found in all samples.

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Appendix

***Appendix Table 1**. Results on fungi (total moulds,* Fusarium*, storage moulds, yeasts; all in cfu/g) and mycotoxins (all in µg/kg) in 42 individual samples of **oats** from different regions 2021. DON=deoxynivalenol, 3-Ac-DON=3-acetyl-DON, 15-Ac-DON=15-acetyl-DON, T-2=T-2 toxin, HT-2=HT-2 toxin, sum of T-2 and HT-2, NIV=nivalenol, ZEA=zearalenone.*

ID-nr.	Total mould	*Fusarium*	Storage mould	Yeast	DON	3-Ac-DON	15-Ac-DON	T-2	HT-2	T-2 + HT-2	NIV	ZEA
OATS Region Øst												
2021-23-242-2	120000	1000	17000	620000	31	<10	<10	51	55	106	59	<3.0
2021-23-243-2	480000	2500	5000	1200000	327	36	<10	76	221	297	60	<3.0
2021-23-244-2	330000	3000	50	360000	42	<10	<10	62	180	242	39	<3.0
2021-23-245-2	130000	5000	150	1100000	187	12	<10	111	170	281	21	<3.0
2021-23-246-1	160000	2500	500	2200000	1050	91	<10	24	24	48	22	<3.0
2021-23-247-1	210000	2500	<50	910000	820	53	<10	334	659	993	101	<3.0
2021-23-248-2	410000	1000	50	1100000	98	22	<10	119	244	363	82	<3.0
2021-23-277-4	480000	4000	250	350000	94	<10	<10	47	172	219	58	<3.0
2021-23-277-5	320000	10000	50	410000	142	<10	<10	39	158	197	49	<3.0
2021-23-278-2	410000	9000	550	860000	61	<10	<10	52	125	177	61	<3.0
2021-23-286-2	1500000	5000	500	700000	84	<10	<10	78	257	335	34	8.5
2021-23-294-2	3200000	20000	50	2700000	36	<10	<10	64	136	200	75	<3.0
2021-23-294-4	4300000	55000	1500	2700000	55	<10	<10	66	259	325	92	<3.0
2021-23-295-2	520000	10000	50	1400000	49	<10	<10	25	76	101	30	<3.0
2021-23-323-1	140000	9000	550	350000	114	<10	<10	159	395	554	130	<3.0
OATS Region Stor-Oslo												
2021-21-232-2	320000	15000	73000	1100000	99	<10	<10	57	175	232	28	<3.0
2021-21-233-2	1500000	5000	25000	7200000	358	20	<10	5	13	18	33	<3.0
2021-23-201-2	500000	5000	<50	1700000	50	<10	<10	65	179	244	14	<3.0
2021-23-202-2	550000	5000	10000	3100000	43	<10	<10	151	221	372	54	<3.0

2021-23-203-2	300000	14000	200	1600000	48	<10	<10	<10	<10	<20	23	12.6
2021-23-206-2	340000	5000	300	2000000	39	<10	<10	41	106	147	27	<3.0
2021-23-207-2	550000	10000	<50	2100000	57	<10	<10	15	86	101	85	<3.0
2021-23-208-2	730000	3000	7500	2400000	111	<10	<10	68	106	174	<10	<3.0
2021-23-209-2	110000	500	<50	640000	135	<10	<10	<10	<10	<20	<10	<3.0
2021-23-221-2	200000	9000	<50	860000	247	18	<10	50	108	158	51	<3.0
2021-23-230-1	590000	4000	<50	1000000	104	<10	<10	21	43	64	11	3.3
2021-23-231-1	240000	3000	300	1200000	87	<10	<10	11	46	57	48	<3.0
2021-23-234-1	440000	5000	1000	680000	82	<10	<10	93	186	279	79	<3.0
2021-23-250-2	1300000	20000	50	1800000	324	17	<10	24	104	128	365	<3.0
2021-23-251-2	910000	5000	50	910000	319	10	<10	12	50	62	83	<3.0
2021-23-264-1	550000	20000	<50	1200000	486	46	<10	<10	<10	<20	15	<3.0
2021-23-265-1	150000	4000	150	730000	106	<10	<10	18	51	69	33	<3.0
2021-23-266-1	440000	2500	250	1000000	162	10	<10	26	82	108	98	<3.0
2021-23-302-2	300000	5000	<50	1300000	201	16	<10	272	592	864	47	<3.0
OATS Region Midt												
2021-23-239-2	55000	5000	5000	27000	383	22	<10	44	105	149	97	<3.0
2021-23-239-4	180000	2500	20000	130000	119	<10	<10	18	55	73	15	<3.0
2021-23-312-1	200000	100000	500	270000	88	<10	<10	37	48	85	294	<3.0
2021-23-312-2	500000	100000	<50	50000	632	83	<10	52	86	138	33	17.5
2021-23-313-1	150000	20000	2500	200000	570	64	<10	11	12	23	75	77.0
2021-23-314-2	700000	20000	<50	200000	1191	181	<10	43	51	94	19	101.0
2021-23-314-4	1200000	9000	10100	1200000	234	14	<10	92	103	195	48	<3.0
2021-23-322-1	73000	500	8000	280000	158	13	<10	11	17	28	77	<3.0

***Appendix Table 2**. Results on fungi (total moulds,* Fusarium*, storage moulds, yeasts; all in cfu/g) and mycotoxins (µg/kg) and Claviceps purpurea (mg/kg) in **barley** based on 47 individual samples from different regions in 2021. DON=deoxynivalenol, 3-Ac-DON=3-acetyl-DON, 15-Ac-DON=15-acetyl-DON, T-2=T-2 toxin, HT-2=HT-2 toxin, NIV=nivalenol, ZEA=zearalenone.*

ID-nr.	Total mould	*Fusarium*	Storage mould	Yeast	DON	3-Ac-DON	15-Ac-DON	T-2	HT-2	NIV	ZEA	Ergo-novine	Ergo-sine	Ergot-amine	Ergo-cornine	α-ergo-criptine	Ergo-cristine	*C. purpurea* sclerotia
BARLEY Region Øst																		
2021-23-242-1	160000	2500	7500	910000	49	<10	<10	<10	13	<10	14.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	3.7
2021-23-243-1	190000	3000	500	1500000	70	<10	<10	109	102	56	<3.0	<2.0	52.0	<2.0	<2.0	2.2	66.0	4.8
2021-23-244-1	25000	4000	50	360000	27	<10	<10	50	44	<10	<3.0	<2.0	41.0	<2.0	<2.0	<2.0	39.0	2.2
2021-23-245-1	60000	3000	500	540000	219	12	<10	<10	<10	12	<3.0	2.4	30.0	<2.0	<2.0	<2.0	20.0	4.5
2021-23-277-1	120000	5000	100	1300000	67	<10	<10	29	72	<10	<3.0	<2.0	14.3	<2.0	<2.0	<2.0	25.3	2.2
2021-23-277-2	170000	5000	<50	2900000	48	<10	<10	<10	<10	<10	<3.0	2.6	23.9	<2.0	11.7	3.4	26.8	15.8
2021-23-277-3	260000	2000	200	2500000	73	<10	<10	<10	<10	<10	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	0.0
2021-23-278-1	300000	27000	1000	2500000	54	<10	<10	10	32	10	<3.0	<2.0	<2.0	2.4	<2.0	<2.0	8.8	20.2
2021-23-286-1	95000	5000	500	770000	48	<10	<10	<10	21	14	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	0.0
2021-23-294-1	250000	10000	130000	1000000	123	<10	<10	28	12	50	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	0.0
2021-23-294-3	290000	10000	40000	1100000	248	<10	<10	<10	42	62	6.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	35.8
2021-23-295-1	290000	5000	500	950000	58	<10	<10	10	23	26	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	0.0
2021-23-305-1	100000	5000	<50	1300000	33	<10	<10	<10	23	<10	<3.0	<2.0	163.8	<2.0	<2.0	<2.0	111.2	52.2
BARLEY Region Stor-Oslo																		
2021-21-232-1	130000	5000	1000	910000	30	<10	<10	55	85	23	<3.0	86.0	28.6	<2.0	328.0	102.0	<2.0	68.4
2021-21-233-1	950000	7500	5000	6200000	45	<10	<10	49	77	27	<30	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	0.0
2021-23-201-1	450000	15000	100	2700000	38	<10	<10	<10	<10	<10	<3.0	<2.0	53.9	<2.0	61.8	22.3	<2.0	5.7
2021-23-202-1	250000	30000	<50	3200000	119	<10	<10	<10	<10	103	<3.0	<20	<2.0	<2.0	<2.0	<2.0	<2.0	7.7
2021-23-203-1	150000	5000	500	2500000	40	<10	<10	<10	12	<10	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	0.0
2021-23-206-1	200000	15000	100	1800000	29	<10	<10	11	40	<10	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	0.0
2021-23-207-1	110000	23000	<50	2600000	44	<10	<10	<10	25	11	<30	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	0.0
2021-23-208-1	500000	45000	250	3500000	329	16	<10	<10	<10	14	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.2
2021-23-209-1	130000	500	500	420000	24	<10	<10	<10	<10	<10	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	0.0
2021-23-220-1	200000	3500	5000	2300000	20	<10	<10	<10	<10	<10	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	7.8
2021-23-221-1	180000	10000	1500	730000	106	<10	<10	<10	27	11	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	1.2
2021-23-234-3	350000	23000	<50	2300000	182	<10	<10	16	33	43	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	46.3

2021-23-250-1	130000	15000	<50	1300000	120	<10	<10	<10	24	31	<3.0	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	0.0
2021-23-251-1	130000	32000	1500	770000	175	<10	<10	<10	10	36	<3.0	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	0.0
2021-23-264-2	110000	5000	50	330000	56	<10	<10	<10	<10	<10	<3.0	<2.0	29.3	<2,0	<2.0	9.3	6.1	0.0
2021-23-265-2	40000	5000	1500	820000	56	<10	<10	<10	11	23	<3.0	<2.0	6.5	<2,0	<2.0	6.3	<2.0	0.0
2021-23-266-2	330000	10000	3500	2400000	291	13	<10	<10	21	43	6.3	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	0.0
2021-23-302-1	1300000	150000	25000	1600000	169	14	<10	109	288	156	31.0	4.4	8.4	<2,0	4.9	3.1	13.3	0.0
2021-23-303-1	260000	5000	31000	1400000	31	<10	<10	32	60	15	<3.0	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	0.0
BARLEY Region Midt																		
2021-23-239-1	140000	5000	1000	640000	18	<10	<10	<10	<10	<10	<3.0	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	0.0
2021-23-239-3	150000	3000	500	910000	26	<10	<10	<10	<10	<10	<3.0	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	2.2
2021-23-288-1	100000	10000	1000	820000	83	<10	<10	<10	<10	<10	3.3	<2.0	149.9	2,8	<2.0	<2.0	85.1	0.0
2021-23-288-2	16000	500	500	900000	<10	<10	<10	<10	<10	<10	<3.0	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	0.0
2021-23-312-3	91000	30000	500	240000	240	12	<10	38	<10	<10	3.7	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	0.0
2021-23-312-4	13000	1000	<50	95000	28	<10	<10	12	20	<10	<3.0	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	0.0
2021-23-313-2	770000	100000	100000	860000	431	25	<10	<10	<10	13	15.2	<2.0	24.8	31,9	<2.0	13.4	40.0	22.0
2021-23-313-3	160000	15000	5000	210000	222	13	<10	<10	<10	12	41.9	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	0.0
2021-23-314-1	280000	5000	50	2000000	29	<10	<10	<10	23	43	<3.0	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	0.0
2021-23-314-3	350000	150000	2500	450000	64	17	<10	<10	<10	<10	104.7	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	0.0
BARLEY Region Sør og Vest																		
2021-23-215-1	500000	5000	350000	200000	94	<10	<10	<10	<10	<10	<3.0	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	0.0
2021-23-215-2	640000	10000	550000	41000	18	<10	<10	<10	<10	16	<3.0	<2.0	<2.0	<2,0	<2.0	<2.0	<2.0	2.5
2021-23-235-1	59000	6500	1000	130000	77	<10	<10	<10	<10	20	4.0	<2.0	<2.0	<2,0	<2.0	<2.0	2.3	19.4
2021-23-235-2	210000	5000	16000	160000	38	<10	<10	<10	<10	<10	<3.0	13.5	33.2	<2,0	56.8	35.9	7.6	3.1
2021-23-236-1	110000	4000	25000	500000	34	<10	<10	<10	<10	11	<3.0	<2.0	4.1	<2,0	<2.0	<2.0	<2.0	2.5

***Appendix Table 3**. Results on mycotoxins (µg/kg) and Claviceps purpurea (mg/kg) in **ryewheat (triticale)** based on 14 individual samples from different regions in 2021. DON=deoxynivalenol, 3-Ac-DON=3-acetyl-DON, 15-Ac-DON=15-acetyl-DON, T-2=T-2 toxin, HT-2=HT-2 toxin, NIV=nivalenol, ZEA=zearalenone.*

ID-nr.	DON	3-Ac-DON	15-Ac-DON	T-2	HT-2	NIV	ZEA	Ergo-novine	Ergo-sine	Ergot-amine	Ergo-cornine	α-ergo-criptine	Ergo-cristine	*C. purpurea* sclerotia
2021-23-203-3	30	<10	<10	<10	10	11	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	0.0
2021-23-206-3	75	<10	<10	<10	<10	11	<3.0	<2.0	<2.0	<2.0	2.3	2.2	<2.0	3.3
2021-23-209-3	149	<10	<10	<10	<10	<10	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	96.2
2021-23-221-3	<10	<10	<10	15	16	14	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.1
2021-23-233-3	36	<10	<10	14	13	24	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	0.0
2021-23-234-2	32	<10	<10	10	13	<10	<3.0	10.2	12.8	12.1	<2.0	<2.0	56.1	20.3
2021-23-248-1	<10	<10	<10	<10	<10	<10	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	16.7
2021-23-264-3	72	<10	<10	<10	<10	<10	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	23.0
2021-23-265-3	306	<10	<10	<10	<10	<10	4.8	4.2	35.5	<2.0	<2.0	<2.0	21.0	36.7
2021-23-286-3	15	<10	<10	<10	<10	<10	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	24.4
2021-23-286-4	36	<10	<10	<10	<10	<10	<3.0	<2.0	5.1	<2.0	<2.0	<2.0	<2.0	6.8
2021-23-286-5	27	<10	<10	<10	13	<10	<3.0	<2.0	10.7	<2.0	<2.0	<2.0	<2.0	27.0
2021-23-295-3	87	<10	<10	10	13	11	<3.0	2.9	3.3	<2.0	12.3	3.8	<2.0	0.0
2021-23-301-1	57	<10	<10	<10	11	<10	<3.0	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	2.1

Appendix Table 4. *Results on mycotoxins in individual samples of* ***complete feed for pigs*** *(20 samples) 2021. All concentrations in µg/kg. DON=deoxynivalenol, 3-Ac-DON=3-acetyl-DON, 15-Ac-DON=15-acetyl-DON, T-2=T-2 toxin, HT-2=HT-2 toxin, NIV=nivalenol, ZEA=zearalenone, OTA=ochratoxin A.*

ID-nr.	Type of feed	DON	3-ac-DON	15-ac-DON	T-2	HT-2	NIV	ZEA	OTA
2021-21-23-1	Format Purke	192	14	<10	29	58	45	15.4	0.1
2021-21-24-1	Opti Norm Energi Våt	130	<10	<10	<10	<10	27	25.7	0.3
2021-21-25-1	Opti Norm Energi Tørr	249	15	<10	11	26	24	25.2	0.2
2021-21-28-1	Optinorm	127	<10	<10	16	40	83	<3.0	0.8
2021-21-42-1	Format vekst 120	83	<10	<10	<10	20	<10	<3.0	0.3
2021-21-42-2	Format Flex 110	110	<10	<10	20	47	16	<3.0	<0.1
2021-21-42-3	Format Drektig	70	<10	<10	<10	38	11	<3.0	0.2
2021-21-43-1	Format Kvikk	95	<10	<10	<10	23	12	<3.0	1.4
2021-21-44-1	Opti Norm Våt Energi	106	<10	<10	<10	23	13	<3.0	<0.1
2021-21-44-2	Opti Norm Våt	102	<10	<10	10	29	16	7.1	<0.1
2021-21-44-3	Opti Vital Prestart	91	<10	<10	<10	14	<10	<3.0	0.1
2021-21-52-1	Batchnr 5720	191	10	<10	<10	12	27	16.5	<0.1
2021-21-79-1	Svin Opti norm	57	<10	<10	16	42	<10	<3.0	<0.1
2021-21-79-2	Svin Opti Smågris	48	<10	<10	<10	17	<10	<3.0	0.1
2021-21-101-1	Drøv fiber storfe	270	<10	<10	16	53	17	24.2	0.4
2021-21-101-2	IDEAL 30	121	<10	<10	14	29	10	11.0	0.5
2021-21-106-1	Format vekst 120	86	<10	<10	43	112	19	3.4	<0.1
2021-21-110-1	Format Purke	87	<10	<10	34	93	19	<3.0	<0.1
2021-21-111-1	Format kvikk 1	44	<10	<10	24	60	15	7.7	<0.1
2021-21-112-1	Format vekst 120	66	<10	<10	17	44	29	<3.0	<0.1

Appendix Table 5*. Results on fungi (total mould,* Penicillium *spp.,* Aspergillus *spp.,* Mucorales*, yeasts; all cfu/g) and mycotoxins (all µg/kg) in 18 individual samples of* ***farm-mixed feed for pigs*** *in 2021. DON=deoxynivalenol, 3-Ac-DON=3-acetyl-DON, 15-Ac-DON=15-acetyl-DON, T-2=T-2 toxin, HT-2=HT-2 toxin, NIV=nivalenol, ZEA=zearalenone, OTA=ochratoxin A.*

ID-nr.	Total moulds	*Penicillium*	*Aspergillus*	Mucorales	Yeasts	DON	3-Ac-DON	15-Ac-DON	T-2	HT-2	NIV	ZEA	OTA
2021-23-139-1	500	450	50	<50	610000	21	<10	<10	<10	<10	<10	<3.0	0.1
2021-23-140-1	8200	50	<50	<50	5000000	22	<10	<10	<10	<10	<10	<3.0	<0.1
2021-23-142-1	5900	4500	<50	500	200000	10	<10	<10	<10	<10	<10	3.5	<0.1
2021-23-143-1	100	<50	<50	<50	1500	25	<10	<10	<10	<10	<10	<3.0	0.1
2021-23-144-1	500	500	<50	<50	1200000	56	<10	<10	<10	15	12	<3.0	<0.1
2021-23-154-1	910	230	680	<50	6300	11	<10	<10	<10	<10	<10	<3.0	0.1
2021-23-155-1	<50	<50	<50	<50	6000000	37	<10	<10	<10	<10	<10	3.0	<0.1
2021-23-156-1	<50	<50	<50	<50	18000000	35	<10	<10	<10	<10	<10	3,6	0.1
2021-23-157-1	13000	<50	9100	3600	30000	18	<10	<10	<10	<10	<10	<3.0	0.5
2021-23-158-1	500	50	50	<50	8200	230	13	<10	<10	<10	<10	9.1	<0.1
2021-23-159-1	150	50	50	<50	50000	32	<10	<10	<10	<10	<10	<3.0	<0.1
2021-23-163-1	170000	120000	500	1500	33000000	14	<10	<10	<10	<10	<10	<3.0	0.1
2021-23-169-1	2300	2300	<50	<50	8100000	14	<10	<10	<10	<10	<10	<3.0	<0.1
2021-23-178-1	41000	41000	<50	3500	18000000	<10	<10	<10	<10	<10	<10	<3.0	<0.1
2021-23-306-1	590	590	<50	<50	4000000	14	<10	<10	<10	<10	<10	<3.0	<0.1
2021-23-315-1	680	50	630	<50	35000	20	<10	<10	<10	<10	<10	<3.0	<0.1
2021-23-331-1	27000	1400	5500	<50	100000	14	<10	<10	<10	<10	<10	<3.0	2.0
2021-23-332-1	1500	1100	500	<50	2000000	<10	<10	<10	<10	<10	<10	<3.0	0.1

Appendix Table 6. *Results on aflatoxin B1, B2, G1, G2, ZEA=zearalenone, OTA=ochratoxin A (µg/kg) in 14 individual samples of maize 2021*

ID-nr.	Type	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	ZEA	OTA
2021-21-4-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	<3.0	<0.1
2021-21-6-1	Maize kernels	4.90	0.30	0.20	<0.1	67.3	0.20
2021-21-20-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	<3.0	<0.1
2021-21-21-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	175.4	0.10
2021-21-22-1	Maize gluten	<0.1	<0.1	<0.1	<0.1	<3.0	<0.1
2021-21-26-1	Maize gluten	<0.1	<0.1	<0.1	<0.1	<3.0	<0.1
2021-21-27-1	Maize kernels	2.60	0.10	<0.1	<0.1	<3.0	<0.1
2021-21-31-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	<3.0	<0.1
2021-21-34-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	<3.0	<0.1
2021-21-40-1	Flaked maize	<0.1	<0.1	<0.1	<0.1	<3.0	<0.1
2021-21-103-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	<3.0	<0.1
2021-21-108-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	418.5	<0.1
2021-21-109-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	90.7	<0.1
2021-21-113-1	Maize kernels	<0.1	<0.1	<0.1	<0.1	90.3	<0.1

Healthy Fish

Healthy animals

Safe food

Scientifically ambitious, forward-looking and collaborative- for one health!

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