



The surveillance programme for feed and feed materials in Norway 2020 - Mycotoxins and fungi



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The surveillance programme for feed and feed materials in Norway 2020 - Mycotoxins and fungi

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Summary

The surveillance programme for feed and feed materials in 2020 included selected mycotoxins and fungi in oats, barley and farm-mixed feed for ruminants, selected mycotoxins in compound feed for pigs, and aflatoxins in maize.

In oats, 64 % of the samples had higher total mould counts than the hygienic guidance value, most consisting of field mould, which are eliminated through normal drying process. In 36 % of the samples, *Fusarium* were measured at levels considered of potential health concern when used as animal feed, but this concern was denied in the analyses of mycotoxins. The levels of total moulds and *Fusarium* were normally high and did not differ from the previous years. Storage moulds and yeasts, in contrast, were mostly within the guidance levels and considered normal.

The high levels of *Fusarium* in oats did not correspond with elevated concentrations of mycotoxins such as deoxynivalenol (DON), zearalenone (ZEN) or T-2/HT-2 toxin. Ochratoxin A (OTA) was not detectable and ergot alkaloids were hardly present in oats.

In barley, *Claviceps purpurea* (ergot) was detected in 62 % of the samples, all far below the maximum limit. Ergot alkaloids were present in some samples at low concentrations. There was no significant correlation between ergot and ergot alkaloids. Trichothecenes and ZEN were detected only at low concentrations and OTA was undetectable.

One sample of maize contained aflatoxins, below the maximum limit.

In compound feed for pig, the mycotoxins DON, T-2/HT-2, nivalenol (NIV), ZEN and ergot alkaloids were found in a few samples, all at low concentrations. OTA was not detectable.

Some samples of farm-mixed feed for ruminants contained high levels of yeasts or storage mould, indicating low hygienic quality. T-2/HT-2 and ZEN were present in a few samples at low concentrations, whereas DON, NIV, OTA and ergot alkaloids were undetectable.

Sammendrag

I overvåkingsprogrammet for mykotoksiner og sopp i fôr og fôrråvarer i 2020 ble ulike mykotoksiner og sopp målt i havre, bygg og i gårdsblandet drøvtyggerfôr. Videre ble utvalgte mykotoksiner målt i kraftfôr til gris og aflatoksiner i mais.

I havre inneholdt 64 prosent av prøvene høyere totalt muggtall enn veiledende grense for hygienisk kvalitet. I 36 prosent av prøvene ble *Fusarium* målt over et nivå som kan indikere potensiell fare for helseproblemer ved bruk som fôr, men slik fare ble ikke bekreftet i mykotoksinresultatene. Nivåene av totalmuggsopp og *Fusarium* var ikke unormalt høye og avviker ikke fra de siste foregående årene. Lagringsmuggsopp og gjær hadde normale nivåer stort sett innenfor veiledende grenser.

Det høye nivået av *Fusarium* i havre gjenspeilte ikke høye konsentrasjoner av mykotoksiner som deoksynivalenol (DON), zearalenon (ZEN) eller T-2 / HT-2-toksin. Okratoksin A (OTA) var ikke påvisbart og ergotalkaloider nesten ikke i havre.

Bygg ble undersøkt for *Claviceps purpurea* (meldrøye) som ble påvist i 62 prosent av prøvene - alle langt under maksimumsgrensen. Meldrøyealkaloider var til stede i lave konsentrasjoner i noen prøver. Det var ingen signifikant sammenheng mellom meldrøye og meldrøyealkaloider. Trichothecener og ZEN ble bare påvist i lave konsentrasjoner i bygg og OTA var ikke påvisbart.

I mais inneholdt en prøve aflatoksiner, men nivået var under maksimal grense.

I kraftfôr til gris ble mykotoksinene DON, T-2 / HT-2, nivalenol (NIV), ZEN og meldrøyealkaloider påvist i noen prøver, alle i lave konsentrasjoner. OTA ble ikke påvist.

Noen prøver av gårdsblandet fôr til drøvtyggere inneholdt høyt nivå av gjær eller lagringsmuggsopp som indikerer lav hygienisk kvalitet. T-2/HT-2 og ZEN ble påvist i noen få prøver i lave konsentrasjoner, mens DON, NIV, OTA og meldrøyealkaloider ikke kunne påvises.

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 give good basis for assessments of feed quality, the impact of animal health and human exposure via animal products.

Fungi in cereals are 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*, *Microdochium*, *Cladosporium*, *Acremonium*, *Epicoccum*, *Phoma* and more. In addition, *Claviceps purpurea* (ergot), belong to field fungi.

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.

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 (ZEN).

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 limited available surveillance data, the oestrogenic mycotoxin ZEN produced by the same *Fusarium* species as DON, is not very common 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*, is 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, 4].

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.

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 minimize 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 2020, the surveillance programme for feed consisted of the following samples shown in Table 1.

Table 1: Samples in the surveillance programme for feed 2020.

Matrix	Planned	Sampled and analysed	Analyses
Oats	45	42	Total moulds, <i>Fusarium</i> , storage moulds, yeast, trichothecenes, zearalenone, ergot alkaloids
Barley	45	45	<i>Claviceps purpurea</i> , trichothecenes, zearalenone, ergot alkaloids
Maize/maize products	15	10	Aflatoxins
Complete compound feed for pigs	20	20	Trichothecenes, zearalenone, ochratoxin A, ergot alkaloids
Farm-mixed feed for ruminants	25	9	Total moulds, <i>Penicillium</i> , <i>Aspergillus</i> , yeast, trichothecenes, zearalenone, ochratoxin A, ergot alkaloids

Oats and barley 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 ruminants were sampled throughout the year. To ensure samples were representative, sampling followed EU Regulation 691/2013. Lower numbers of sampled farm-mixed feed for ruminants and maize than planned were mainly due to the restricted work situation during the corona epidemic.

Quantitative determination of fungi in oats and farm-mixed feed for ruminants (ME02_050)

Quantitative determinations of total moulds and yeasts in oats 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 ruminants 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 (ME02_154)

Claviceps purpurea sclerotia in grams per kg cereal were calculated according to the method described by Vrålstad *et al.* [5]. 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, pig feed and ruminant feed

The multi-mycotoxin liquid chromatography-high-resolution mass spectrometry (LC-HRMS/MS) method was used for the simultaneous determination of mycotoxins [6]. 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 27 to 96 % were demonstrated for all selected mycotoxins. Reasonable levels of signal suppression or signal enhancement (70 - 120 %) were achieved for only 20 % of targeted mycotoxins. Therefore, in order to improve the accuracy of the method, stable-isotope labelled internal standards (IS) were introduced for nine of the analysed mycotoxins including DON, and 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, ZEN and OTA. For quantitative analysis of ergot alkaloids, semisynthetic ergot derivatives were used for the preparation of IS calibrations. Statistics from a proficiency test 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 precision in terms of total within laboratory precision ($RSiR(\%) = \sqrt{RSDr^2 + RSDL^2}$) by considering intra and inter day variabilities together (Table 2). By considering the negligible noise in the extracted high-resolution mass chromatograms, the LODs of the targeted mycotoxins were calculated based on the standard deviation of the y-intercept of the respective calibration curves and their corresponding slopes (m) as $LOD = 3 \times SD/m$ (Table 2).

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

Toxin	LOD, $\mu\text{g}/\text{kg}$	Total within laboratory precision (%)				Recovery \pm SD (%)			
		Oats	Barley	Pig feed	Ruminant feed	Oats	Barley	Pig feed	Ruminant feed
Deoxynivalenol	66	8	4	8	11	121 \pm 13	121 \pm 14	118 \pm 13	121 \pm 15
DON-3-glucoside	79	7	12	11	13	103 \pm 19	94 \pm 15	105 \pm 22	103 \pm 24
3-Acetyl-DON	15	7	7	6	4	118 \pm 14	122 \pm 10	123 \pm 9	123 \pm 6
15-Acetyl-DON	52	7	4	11	14	133 \pm 3	134 \pm 14	143 \pm 24	227 \pm 43
Nivalenol	30	8	5	9	16	118 \pm 10	111 \pm 11	110 \pm 18	106 \pm 7
T-2 toxin	13	6	9	8	4	118 \pm 9	119 \pm 6	119 \pm 16	132 \pm 6
HT-2 toxin	22	10	9	13	17	129 \pm 16	122 \pm 11	131 \pm 16	124 \pm 11
Zearalenone	10	10	8	12	6	105 \pm 16	121 \pm 7	109 \pm 19	127 \pm 6
Ochratoxin A	21	7	13	15	27	95 \pm 11	110 \pm 11	97 \pm 15	98 \pm 13
Ergonovine*	55	15	7	24	6	191 \pm 42	116 \pm 8	131 \pm 36	102 \pm 7
Ergosine*	13	15	9	7	16	152 \pm 17	124 \pm 12	117 \pm 14	68 \pm 13
Ergotamine*	39	13	10	9	18	130 \pm 14	110 \pm 10	102 \pm 13	57 \pm 8
Ergocornine*	12	14	12	10	15	152 \pm 22	114 \pm 9	123 \pm 13	65 \pm 7
α -Ergocryptine*	185	17	10	11	17	129 \pm 25	107 \pm 15	109 \pm 18	73 \pm 12
Ergocristine*	24	14	12	18	17	108 \pm 16	103 \pm 14	102 \pm 17	66 \pm 9

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

The extraction methodology was based on the 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 extraction with respect to polar and non-polar compounds.

The LC-HRMS analyses were performed on a Q-Exactive™ Hybrid Quadrupole-Orbitrap mass spectrometer equipped with a heated electrospray ion source (HESI-II) and coupled to a Vanquish UHPLC system (Thermo Scientific). The Q-Exactive HRMS/MS was operated in full scan (FS) mode with the inclusion of targeted fragmentation (data-dependent MS/MS: dd-MS2).

Chemical analysis of maize

Aflatoxins (B1, B2, G1, G2) in maize were analysed using high-performance liquid chromatography with fluorescence (HPLC-FID) and post-column derivatisation. Prior to HPLC-FID injection, clean-up steps involving immune affinity columns (IAC) to remove interferences were required. LOD for aflatoxins were as follow: B1: 0.25 $\mu\text{g}/\text{kg}$, B2: 0.10 $\mu\text{g}/\text{kg}$, G1: 0.20 $\mu\text{g}/\text{kg}$, G2: 0.15 $\mu\text{g}/\text{kg}$.

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, 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) [7] in 64 % of the samples (Table 3), indicating poor hygienic quality if used directly. In general, feed of poor hygienic quality can cause reduced growth rate and create health problems in animals [8]. 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. The level of total moulds in oats 2020 is considered at a “normally high” level which varied little from those in 2018 and 2019 [3, 9].

Fusarium spp., detected in 98 % of the oat samples, had levels above 25,000 cfu/g in 36 % of the samples, which due to mycotoxin production, would be potentially hazardous if used as feed [7]. The *Fusarium* level for oats in 2020 is considered at “normally high” levels, which were somewhat lower than in 2019 but higher than in 2018, with mean and median above 2 times higher in 2020 compared with 2018 [3, 9].

Storage moulds were found in 50 % of the oat samples, and 5 % exceeded the guidance value for storage moulds at 100,000 cfu/g [7]. The level of storage moulds in 2020 was relatively similar as in 2018 - much higher than the extraordinarily low levels in 2019 [3, 9].

Yeasts were detected in all samples, but none exceeded the guidance value for yeasts at 10,000,000 cfu/g [7]. The levels of yeasts in 2020 for oats varied little from levels in 2019 and 2018 [3, 8].

Table 3: Occurrence of fungi (cfu/g of total *Fusarium* spp., storage moulds and yeasts) and mycotoxins ($\mu\text{g}/\text{kg}$ of trichothecenes (deoxynivalenol (DON), 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON), DON-3-glucoside (DON-3-G), sum of HT-2 and T-2 toxin, nivalenol (NIV)) and zearalenone (ZEN)) in oats (N = 42) sampled in Norway in 2020.

	Total moulds	<i>Fusarium</i> spp.	Storage moulds	Yeasts	DON	3-Ac-DON	15-Ac-DON	DON-3-G	T-2	HT-2	T-2+HT-2	NIV	ZEN
Mean	732 000	26 100	32700	647 100	223	52	<52	<80	47	123	170	70	10
Median	670 000	19 000	<50	595 000	143	44	<52	<80	26	90	132	<30	<10
Minimum	50	<50	<50	50	<66	<15	<52	<80	<13	<22	<35	<30	<10
Maximum	2 000 000	100 000	590 000	2 000 000	955	355	126	372	254	423	615	443	139
SD*	454 200	24 600	106 300	458 300	211	56	15	52	53	108	154	92	22
% samples >dl*	100	98	50	100	83	81	2	2	71	81	81	50	10
% samples >gv*	64	36	5	0	0						5		0

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

In 2020, the levels of the various groups of fungi in the oat samples were rather independent as none of the groups were significantly correlated (Table 4). This result is in contrast to findings from previous years, where at least total moulds and *Fusarium* were positively correlated [3, 4, 9].

Table 4: Correlation coefficients between counts of the various groups of fungi in oats (N = 42) sampled in Norway in 2020. None were significantly correlated ($p > 0.05$).

	Total moulds	<i>Fusarium</i> spp.	Storage moulds	Yeasts
Total moulds	1.000			
<i>Fusarium</i> spp.	0.201	1.000		
Storage moulds	0.058	0.164	1.000	
Yeasts	0.181	0.152	-0.193	1.000

The frequencies of the dominating *Fusarium* species found in oats are presented in Table 5. Similar to 2019 and 2018, *Fusarium poae* was the main species in 2020 [3, 9]. In addition, *F. langsethiae* and *F. graminearum* were similarly present these three years. *F. poae* may produce NIV and ZEN and other secondary fungal metabolites but not DON [10]. *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 less important mycotoxins such as moniliformin, enniatins and others [1]. Occurrence of the *Fusarium* species does not necessarily mean occurrence of toxins as stressors for toxin production may not be present or the cereal plant has implemented toxin preventive mechanisms [11].

Table 5: The frequency of the dominating *Fusarium* species found in oats (N = 42) sampled in Norway in 2020.

Species	Number (%) of samples detected	Number of samples with most dominant species
<i>F. poae</i>	27 (64 %)	19
<i>F. avenaceum</i>	17 (40 %)	8
<i>F. langsethiae</i>	15 (36 %)	12
<i>F. graminearum</i>	6 (14 %)	4
<i>F. tricinctum</i>	3 (7 %)	0
<i>F. culmorum</i>	2 (5 %)	2

In 2020, DON and DON-related compounds in oats were detected in most samples but at low concentrations (Table 3), similarly as in 2017-2019 [3, 4, 9]. All samples had levels far below the limit for DON recommended by EU and Norway (8000 µg/kg) [7, 12].

The DON-related compounds included in the analysis of oats were the acetylated precursor compounds (3-Ac-DON and 15-Ac-DON) and a glucoside metabolite (DON-3-G). 3-Ac-DON was found at low concentrations in most samples, whereas 15-Ac-DON and DON-3-G were hardly present. DON and 3-Ac-DON were significantly positively correlated (Figure 1). 3-Ac-DON was present at about 20 % level compared to DON, which is more than the average in European cereals (about 10 % compared to DON) [13]. However, with almost absence of 15-Ac-DON and

DON-3-G, the total contribution of related compounds in the Norwegian oats were below the average 45 % level compared to DON in Europe [13].

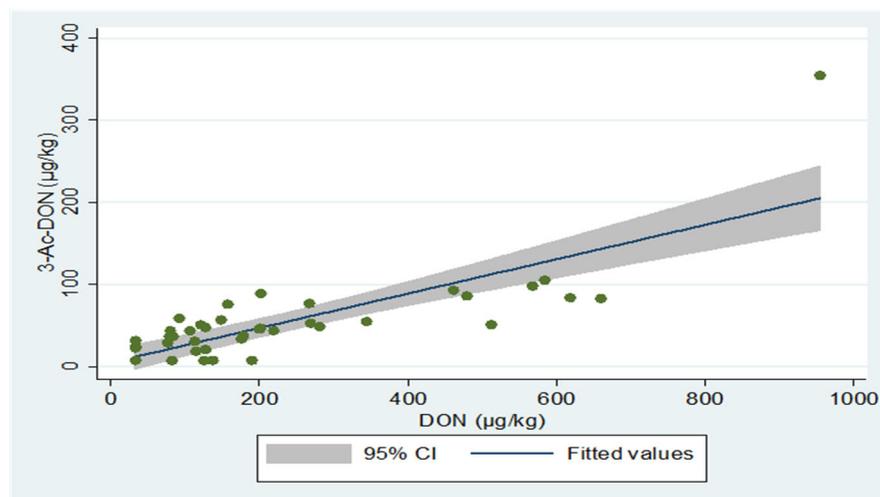


Figure 1: The Pearson correlation (r) between deoxynivalenol (DON) and 3-acetyl-DON in oats ($N=42$) was 0.798, $p<0.0001$. A regression line with 95 % confidence interval fitted to the points allows predictions of levels of 3-Ac-DON given the level of DON detected and vice versa.

In addition, T-2 and HT-2 were present in most oat samples but at generally low concentrations. Two samples (5 %) showed the combined T-2+HT-2 concentrations numerically above the guidance level of 500 $\mu\text{g}/\text{kg}$ in EU and Norway [7, 14] (Table 3; Figure 2). However, the two samples were not regulatory exceedance of the limit due to uncertainty of the analytical method. The mean concentration of T-2+HT-2 was not different from 2016-18, and somewhat lower than in 2019 [3, 4, 9, 15]. T-2 and HT-2 were highly correlated in oats, with an average concentration of HT-2 twice that of T-2 (Figure 2).

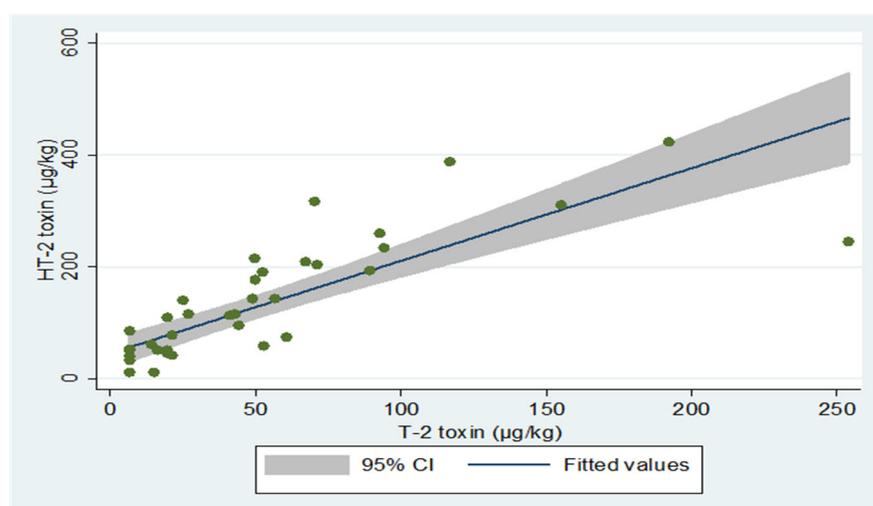


Figure 2: The Pearson correlation (r) between T-2 toxin and HT-2 toxin in oats ($N=42$) was 0.815 ($p<0.0001$). A regression line with 95 % confidence interval fitted to the points allows predictions of levels of HT-2 toxin given the level of T-2 toxin detected and vice versa.

The concentrations of DON and T-2 + HT-2 in oats have been determined in surveillance programs since 2002. The mean DON concentration in 2020 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. Figure 3 also shows that the mean concentration of T-2+HT-2 in 2020 was relatively low.

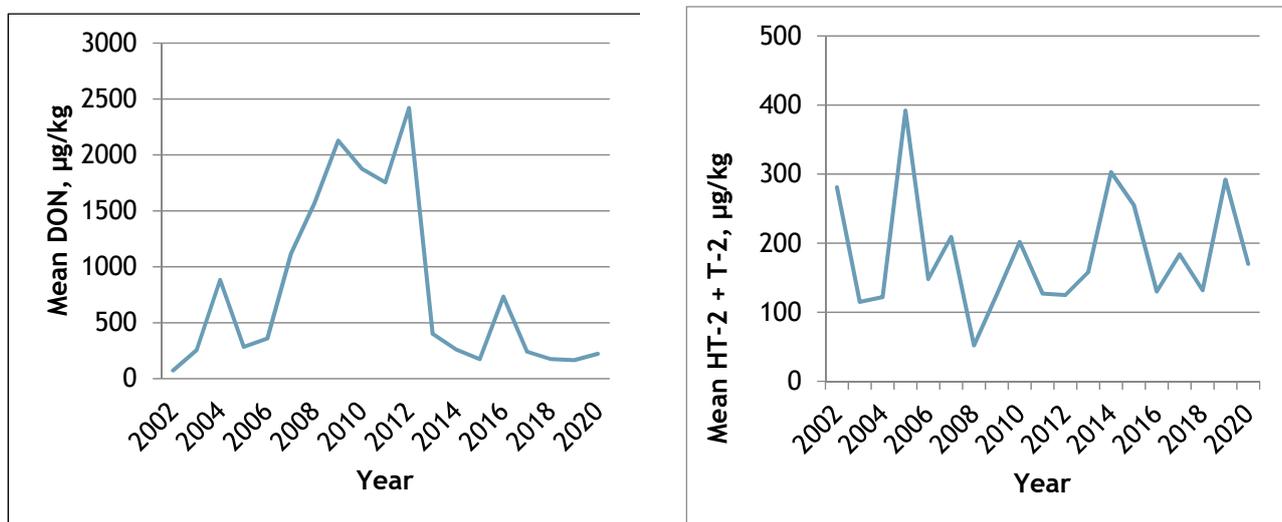


Figure 3: Mean concentration of deoxynivalenol (DON) (left) and of the sum of T-2 toxin and HT-2 toxin (right) in 30-60 samples of oats per year in the Norwegian surveillance programme for feed.

NIV was detectable in 50 % of the oat samples (Table 3). The much lower occurrence of NIV than DON is as usual in Norway. In addition, ZEN, detected in a few samples, were in trace amounts, and far below the recommended limit (2000 µg/kg) in EU and Norway (Table 3) [7, 12]. OTA was not detectable in any sample (below 21 µg/kg).

Concerning ergot alkaloids, only ergosine and ergocornine were detectable at trace amounts in single oat samples (Appendix table 1). Insignificant or not detectable concentrations of ergot alkaloids are common findings for oats.

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 2020 was cold and humid in cereal producing regions in Norway [16]. August and September were relatively warm, with low precipitation in August but somewhat above the normal level in September.

The pattern of moulds and mycotoxins in 2020 showed “normally high” levels of total moulds and *Fusarium*, average levels of storage moulds and yeasts and low concentrations of DON and T-2/HT-2 compared with previous years. The low temperature together with humid conditions in July are probably the basis for the relative high mould and *Fusarium* levels without influence of producing DON and T-2/HT-2. The weather during late summer and autumn facilitated good harvesting conditions with low toxin concentrations.

Fungi and mycotoxins in oats were analysed for regional differences. Storage moulds were significantly higher in region Sør-Vest than in the other regions. No other differences between regions were found.

Table 6: Survey between regions Øst (Buskerud, Vestfold, Telemark, Innlandet), Stor-Oslo (Akershus, Oslo, Østfold), Midt (Trøndelag, Møre, Romsdal) and Sør og Vest (Agder, Rogaland, Vestland) 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 (ZEN); all toxin concentrations µg/kg) in oats (N = 42) sampled in Norway in 2020. Variables that were significantly different between regions are indicated by an * ($p < 0.05$).

Region		Total moulds	<i>Fusarium</i> spp.	Storage moulds*	Yeasts	DON	T-2+ HT-2	NIV	ZEN
Øst n=15	Mean	748 000	20 900	1 300	909 300	161	228	75	<10
	St. dev.	422 600	24 500	2 100	409 700	146	187	118	0
Stor-Oslo n=18	Mean	812 800	26 800	21 800	549 400	198	152	69	<10
	St. dev.	362 700	21 000	29 200	435 500	174	112	66	13
Midt n=7	Mean	485 200	29 000	<50	457 200	393	51	76	26
	St. dev.	727 100	35 300	9	440 600	347	45	107	50
Sør og Vest n=2	Mean	750 000	47 500	480 000	225 000	313	305	<30	<10
	St. dev.	28 300	3 540	155 600	247 500	45	274	0	0

Fungi and mycotoxins in barley

Claviceps purpurea, the only fungus included in the surveillance of barley in 2020, was detected in 62 % of the samples. The ergot had an overall mean level of 19 mg/kg and a maximum of 223 mg/kg (Table 7). Thus, all samples were far below the maximum concentration of 1000 mg/kg [7]. Much of the ergot sclerotia were small in size, indicating they had been growing on grass. Only eight samples (18 %) had sclerotia of the same size or bigger than the barley grain/kernel, indicating that the ergot had grown on the barley.

Ergot alkaloids were present in seven samples (16 %) at maximum concentration of alkaloids 1010 µg/kg. Ergosine and ergocristine were the most common alkaloids with maximum concentrations respectively 454 and 408 µg/kg, found in the same sample (Table 7). 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, sum alkaloids were within 2200 and 3000 µg/kg in 2016, -17 and -19 [3, 4, 10, 15]. The occurrence of alkaloids was too sparse to look for regional differences.

Table 7: Occurrence of *Claviceps purpurea sclerotia* (mg/kg) and ergot toxins ($\mu\text{g/kg}$) consisting of ergotamine/ergotaminine, ergocornine/ergocorninine, α -ergocryptine/ α -ergocryptinine, ergocristine/ergocristinine and sum ergot alkaloids in barley ($N = 45$) sampled in Norway in 2020.

	<i>C. purpurea sclerotia</i>	Ergo-novine/-inine	Ergo-sine/-inine	Ergot-amine/-inine	Ergo-cornine/-inine	α -Ergo-cryptine/-inine	Ergo-cristine/-inine	Σ Ergot alkaloids
Mean	19	<56	20	<40	<12	<190	25	<332
Median	2	<56	<12	<40	<12	<190	<24	<332
Minimum	0	<56	<12	<40	<12	<190	<24	<332
Maximum	223	<56	454	143	37	<190	408	1010
SD*	44	0	67	18	5	0	61	132
% samples >dl*	62	0	13	2	2	0	9	4

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

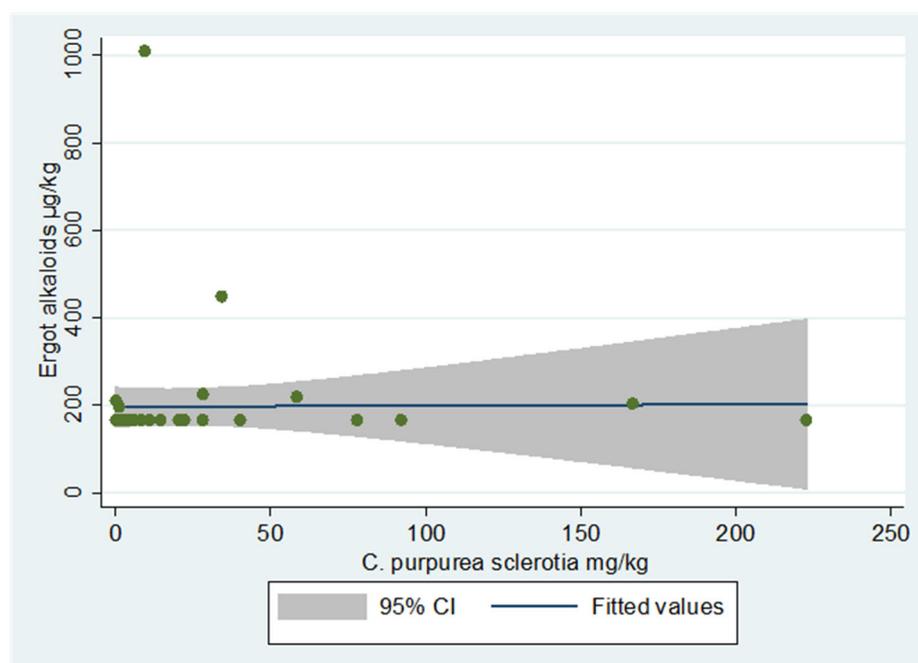


Figure 4: The Pearson correlation (r) between *Claviceps purpurea* and sum of ergot alkaloids detected (ergosine, ergocristine, ergotamine, ergocornine) in samples of barley ($n = 45$) was 0.010 ($p=0.946$). A regression line fitted to the points with 95 % confidence interval is shown.

There was no significant correlation between the ergot and the ergot alkaloids in barley (Figure 5). Lack of correlations between ergot and alkaloids were also found in previous surveys in Norway, in barley in 2019, and in rye and wheat in 2016 and 2017 [3, 17, 18]. These results indicate variable production of alkaloids by the ergot fungi.

Results on trichothecenes and ZEN in barley, as presented in Table 8 show that concentrations were as usual lower than those found in oats. OTA was not detected in barley.

Table 8: Occurrence ($\mu\text{g}/\text{kg}$) of trichothecenes (deoxynivalenol (DON), 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON), DON-3-glucoside (DON-3-G), T-2 toxin, HT-2 toxin, sum of T-2 and HT-2, nivalenol (NIV)) and zearalenone (ZEN) in barley ($n = 45$) sampled in Norway in 2020.

	DON	3-Ac-DON	15-Ac-DON	DON-3-G	T-2	HT-2	T-2+HT-2	NIV	ZEN
Mean	114	<15	<52	<80	<13	<22	<35	<30	<10
Median	68	<15	<52	<80	<13	<22	<35	<30	<10
Minimum	<66	<15	<52	<80	<13	<22	<35	<30	<10
Maximum	621	82	<52	178	32	57	64	115	69
SD*	127	14	0	30	4	9	11	19	14
% samples >dl*	53	20	0	16	5	13	11	29	16
% samples >gv*	0						0		0

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

Aflatoxins in maize

Aflatoxins were detectable in one of ten analysed maize samples, with concentrations of aflatoxin B1 and B2 detected at 5.31 and 0.50 $\mu\text{g}/\text{kg}$, respectively. Noticeably, the positive maize gluten sample did not exceed the maximum limit of aflatoxin B1 (20 $\mu\text{g}/\text{kg}$) [20].

Feed

Feed for pigs

The results on mycotoxins in complete compound feed for pigs in Table 9 show that DON was detected in eight samples (40 %). All were below the recommended limit of DON for pig feed in Norway (500 $\mu\text{g}/\text{kg}$) [7]. Co-occurrence of DON-related compounds were mostly undetectable, with only trace amounts of 3-Ac-DON in two 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 [13].

The sum of T-2 and HT-2 was present in seven (35 %) samples of the pig feed without exceeding the recommended limit (250 $\mu\text{g}/\text{kg}$) [7, 14]. The concentrations of DON and T-2/HT-2 in pig feed in 2020 were similar to results from 2018 and 2019, but lower than 2016 and 2017 [3, 4, 9, 15].

ZEN was present at low concentrations in four samples, all below the recommended limit for pig feed in Norway (250 $\mu\text{g}/\text{kg}$) [7]. These results have remained unchanged in the last years.

Only trace amounts of NIV were detectable in some samples, and OTA was not detectable in feed for pigs.

Ergot alkaloids, ergosine and ergocristine, were found at low concentrations in three samples. Maximum sum of these alkaloids was 130 $\mu\text{g}/\text{kg}$. In 2019, maximum ergot alkaloids was 664 $\mu\text{g}/\text{kg}$ [3]. Such compounds were not detectable in pig feed in 2018 and 2016, but were found in 2017 in a single sample at a total alkaloid concentration of 1620 $\mu\text{g}/\text{kg}$ [4, 9, 15].

Table 9: Concentrations of deoxynivalenol (DON), 3-acetyl-DON, 15-acetyl-DON, DON-3-glucoside, T-2, HT-2, sum of T-2 and HT-2 toxin, nivalenol (NIV), zearalenone (ZEN) and sum of ergot alkaloids ($\mu\text{g}/\text{kg}$) in complete compound feed for pigs ($N = 20$) sampled in Norway in 2020.

	DON	3-Ac-DON	15-Ac-DON	DON-3-G	T-2	HT-2	T-2+HT-2	NIV	ZEN	Σ Ergot alkaloids
Mean	71	<15	<52	<79	<13	23	<35	<30	<10	<332
Median	<66	<15	<52	<79	<13	<22	<35	<30	<10	<332
Minimum	<66	<15	<52	<79	<13	<22	<35	<30	<10	<332
Maximum	199	51	<52	<79	31	66	97	48	21	<332
SD*	57	11	0	0	6	16	21	12	4	30
% samples >dl*	40	10	0	0	10	45	35	20	20	0
% samples >gv*	0						0		0	

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

Feed for ruminants

Complete feed for ruminants mixed at the farm were analysed for total moulds, specific genera of toxigenic storage moulds (*Penicillium*, *Aspergillus*) and yeasts, as well as trichothecenes, ZEN, OTA and ergot alkaloids (Table 10).

Table 10: Occurrence of total moulds, *Penicillium* spp., *Aspergillus* spp., yeasts (cfu/g), and mycotoxins ($\mu\text{g}/\text{kg}$) of trichothecenes (deoxynivalenol (DON), 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON), DON-3-glucoside (DON-3-G), sum of HT-2 and T-2 toxin, nivalenol (NIV)) and zearalenone (ZEN) in samples of farm-mixed feed for ruminants ($N = 9$) sampled in Norway in 2020.

	Total moulds	<i>Penicillium</i>	<i>Aspergillus</i>	Yeasts	DON	3-Ac-DON	15-Ac-DON	DON-3-G	T-2+HT-2	NIV	ZEN
Mean	10 186	7 700	1 700	9 803 500	<66	<15	<52	<80	<35	<30	17
Median	2 500	300	<50	83 000	<66	<15	<52	<80	<35	<30	<10
Minimum	<50	<50	<50	<50	<66	<15	<52	<80	<35	<30	<10
Maximum	45 000	33 000	15 000	55 000 000	<66	<15	<52	<80	42	<30	102
SD*	16 800	12 500	5 000	18 781 800	0	0	0	0	8	0	32
% samples >dl*	89	78	11	89	0	0	0	0	11	0	22
% samples >gv*	0	0	0	22	0				0		0

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

Most of the fungi growth consisted of storage moulds in the genera *Penicillium*, *Aspergillus* and *Mucor*. Some of the samples were frozen before submission, which may have reduced levels of viable fungi. *Penicillium* spp., found in seven samples (78 %), did not exceed the guidance value (50,000 cfu/g) [7] (Table 10). However, in two samples the occurrence of *Penicillium* spp. were between 10,000 - 50,000 cfu/g indicating somewhat reduced hygienic quality [7]. The dominating *Penicillium* species was *P. roqueforti* which is a potential producer of the mycotoxins roquefortine C, PR-toxin, mycophenolic acid and isofumigaclavine A & B. In the multi-mycotoxin method these mycotoxins are not included.

Aspergillus spp. exceeded the detection limit in one sample (11 %), not above the guidance value (50,000 cfu/g) [7].

Yeasts, found in eight samples (89 %), had two samples (22 %) exceeding guidance value (10,000,000 cfu/g) indicating poor hygienic quality [7]. In addition, two samples (22 %) had occurrence of yeasts between 1,000,000 - 10,000,000 cfu/g), indicating reduced hygienic quality.

Trichothecenes and ZEN were hardly detected, and OTA and ergot alkaloids not detectable in the farm-mixed feed for ruminants.

Conclusions

Feed materials

- **Fungi in oats:** In 64 % of the samples, total mould counts were higher than the hygienic guidance value. Most consisting of field moulds which are eliminated through normal drying process. In 36 % of the samples, *Fusarium* were measured at levels considered of potential health concern, but this concern was denied in the analyses of mycotoxins (see below). The dominating *Fusarium* species were *F. poae*, *F. avenaceum* and *F. langsethiae*. Total moulds and *Fusarium* were considered as “normally high” levels. Levels of yeasts and storage moulds were mostly within the guidance levels and considered normal. Regional difference with significantly higher levels for storage moulds was observed in region Sør-Vest compared with other regions.
- **Mycotoxins in oats:** The high levels of *Fusarium* spp. did not correspondingly reflect elevated concentrations of mycotoxins such as DON, ZEN and T-2/HT-2. Levels of DON and ZEN were low and those of T-2 and HT-2 were more normal, with two samples above the guidance level. Ochratoxin A was not detectable and ergot alkaloids were hardly present.
- **Fungi in barley:** *Claviceps purpurea*, detected in 62 % of the samples, were at levels far below the maximum limit at 1000 mg/kg.
- **Mycotoxins in barley:** Ergot alkaloids were present in some samples at low concentrations. No significant correlation between ergot and ergot alkaloids was found. Trichothecenes and ZEN were only detected at low concentrations and ochratoxin A was not detectable.
- **Aflatoxins in maize:** Aflatoxins were detectable in one out of ten samples. The concentration of aflatoxin B1 was 5.3 µg/kg and did not exceed the maximum limit (20 µg/kg).

Feed

- **Compound feed for pig:** The mycotoxins DON, T-2/HT-2, NIV, ZEN and ergot alkaloids were found at low concentrations in a few samples. Ochratoxin A was not detectable.
- **Farm-mixed feed for ruminants:** Some of the samples contained high levels of yeast or storage mould indicating low hygienic quality. T-2/HT-2 and ZEN were present in a few samples at low concentrations, whereas DON, NIV, OTA and ergot alkaloids were undetectable.

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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 2020. 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=niavalenol, ZEN=zearalenone, OTA=ochratoxin A.

ID-nr.	Water%	Total moulds	Fusarium	Storage moulds	Yeasts	DON	3-Ac-DON	15-Ac-DON	DON-3-G	T-2	HT-2	NIV	ZEN	OTA	Ergo-novine	Ergo-sine	Ergot-amine	Ergo-cornine	α-ergo-cryptine	Ergo-cristine
OATS Region Øst																				
2020-23-221-1		1200000	35000	<50	730000	512	51	<52	<79	<13	40	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-222-4		550000	9000	5000	640000	137	<15	<52	<79	43	115	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-222-5		1500000	10000	<50	1700000	<66	<15	<52	<79	155	311	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-222-6		730000	15000	2400	910000	<66	<15	<52	<79	93	260	<30	<10	<21	<55	15	<39	<12	<190	<24
2020-23-228-2	14,2	800000	100000	<50	1500000	202	89	<52	<79	94	234	110	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-229-1	15,7	410000	10000	<50	850000	<66	<15	<52	<79	25	140	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-230-2	12,6	1400000	10000	5100	860000	82	<15	<52	<79	27	115	443	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-231-1	12,6	1400000	10000	100	1100000	125	<15	<52	<79	<13	85	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-232-2	20,0	450000	27000	5000	950000	127	21	<52	<79	117	388	124	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-233-2	14,6	160000	5000	1000	640000	190	<15	<52	<79	21	42	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-255-1		630000	10000	<50	310000	121	51	<52	<79	192	423	40	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-256-1		540000	20000	<50	400000	106	44	<52	<79	67	209	227	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-258-2		450000	41000	<50	450000	461	93	<52	<79	<13	<22	31	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-258-4		400000	10000	<50	1200000	219	44	<52	<79	<13	51	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-304-2	11,4	600000	1500	<50	1400000	<66	32	<52	<79	44	95	37	<10	<21	<55	<12	<39	<12	<190	<24
Oats Region Stor-Oslo																				
2020-23-311-2		270000	2000	10000	350000	127	48	<52	<79	89	193	<30	57	<21	<55	<12	<39	<12	<190	<24
2020-23-312-2	14,3	700000	50000	<50	2000000	<66	25	<52	<79	<13	<22	47	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-325-2	13,2	860000	20000	<50	150000	148	57	<52	<79	50	215	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-326-2	12,4	500000	45000	<50	640000	178	38	<52	<79	21	78	134	<10	<21	<55	<12	<39	<12	<190	<24

2020-23-327-2	13,8	230000	90000	<50	820000	92	59	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-347-2	11,1	850000	15000	50000	770000	584	105	<52	<79	71	204	38	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-348-2	14,2	1100000	20000	<50	550000	479	86	<52	<79	52	190	69	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-348-3	14,6	1300000	14000	1050	250000	568	98	<52	<79	49	143	37	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-353-2	14,2	500000	15000	50000	270000	157	76	<52	<79	41	113	88	<10	<21	<55	<12	<39	12	<190	<24
2020-23-354-2	14,7	640000	20000	1000	300000	267	77	<52	<79	57	143	215	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-355-2	14,4	450000	10000	1000	10000	80	44	<52	<79	50	177	142	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-356-2	14,0	1400000	30000	50000	860000	269	53	<52	<79	70	317	215	22	<21	<55	<12	<39	<12	<190	<24
2020-23-364-2	15,1	730000	10000	10000	550000	76	29	<52	<79	<13	33	64	10	<21	<55	<12	<39	<12	<190	<24
2020-23-365-2	13,9	1100000	10000	50000	500000	200	46	<52	<79	16	51	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-366-2	12,9	950000	23000	100000	730000	<66	23	<52	<79	20	45	52	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-367-2	13,9	1300000	27000	50000	360000	79	37	<52	<79	<13	52	46	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-368-2	14,3	550000	50000	<50	230000	83	37	<52	<79	20	109	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-381-2	12,4	1200000	32000	20000	550000	113	31	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
OATS Region Midt																				
2020-23-303-2	Lo	50	<50	50	50	<66	<15	<52	<79	61	74	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-305-2	9,8	2000000	100000	<50	350000	955	355	126	372	<13	<22	184	139	<21	<55	<12	<39	<12	<190	<24
2020-23-308-1	13,0	86000	10000	<50	150000	660	83	<52	<79	14	61	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-369-2	10,8	100000	20000	<50	10000	201	47	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-369-3	16,1	150000	50000	<50	1100000	114	19	<52	<79	20	51	271	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-370-2	14,7	860000	18000	<50	860000	619	84	<52	80	15	<22	<30	21	<21	<55	<12	<39	<12	<190	<24
2020-23-371-1	14,6	200000	5000	<50	730000	175	34	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
OATS Region Sør og Vest																				
2020-23-298-2		730000	45000	590000	400000	281	49	<52	<79	53	58	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-301-2	17,0	770000	50000	370000	50000	344	55	<52	<79	254	245	<30	<10	<21	<55	<12	<39	<12	<190	<24

Appendix Table 2: Results on mycotoxins ($\mu\text{g}/\text{kg}$) and *Claviceps purpurea* (mg/kg) in barley based on 45 individual samples from different regions 2020.
 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, ZEN=zearalenone, OTA=ochratoxin A.

ID-nr.	Water%	DON	3-Ac DON	15-Ac Don	DON-3-G	T-2	HT-2	NIV	ZEN	OTA	Ergo-novine	Ergo-sine	Ergot-amine	Ergo-cornine	α -ergo-cryptine	Ergo-cristine	<i>C. purpurea</i> sclerotia
BARLEY Region Øst																	
2020-23-222-1	13,9	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	6,0
2020-23-222-2	13,4	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-222-3	14,0	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	28,0
2020-23-228-1	13,4	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-230-1	13,6	191	<15	<52	<79	<13	27	<30	<10	<21	<55	<12	<39	<12	<190	<24	22,0
2020-23-232-1	20,0	621	22	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-233-1	17,1	68	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	2,0
2020-23-255-2		<66	<15	<52	<79	<13	<22	31	<10	<21	<55	<12	<39	<12	<190	<24	222,7
2020-23-256-2		203	<15	<52	<79	<13	<22	115	<10	<21	<55	44	<39	<12	<190	<24	166,7
2020-23-258-1		<66	<15	<52	<79	<13	<22	31	<10	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-258-3		120	<15	<52	<79	32	29	36	<10	<21	<55	<12	<39	<12	<190	<24	1,4
2020-23-304-1	13,0	68	<15	<52	<79	14	27	<30	<10	<21	<55	<12	<39	<12	<190	<24	5,1
2020-23-304-3	12,6	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	2,1
BARLEY Region Stor-Oslo																	
2020-23-311-1	14,1	106	<15	<52	<79	<13	<22	42	<10	<21	<55	<12	<39	<12	<190	<24	2,1
2020-23-312-1	12,9	<66	<15	<52	<79	<13	<22	54	<10	<21	<55	<12	<39	<12	<190	<24	3,2
2020-23-325-1	13,5	69	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-326-1	13,4	124	<15	<52	81	<13	<22	57	14	<21	<55	14	<39	<12	<190	64	28,0
2020-23-327-1	16,8	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-347-1	11,1	<66	<15	<52	<79	<13	34	<30	<10	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-348-1	14,1	85	<15	<52	<79	<13	<22	<30	<10	<21	<55	454	<39	<12	<190	408	9,3
2020-23-353-1	13,4	134	<15	<52	<79	<13	29	38	<10	<21	<55	<12	<39	<12	<190	<24	3,3
2020-23-354-1	14,9	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	1,1
2020-23-355-1	14,0	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-356-1	13,9	203	35	<52	82	<13	<22	38	<10	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-364-1	14,6	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	0,9

2020-23-365-1	14,2	216	16	<52	<79	<13	<22	46	14	<21	<55	50	<39	<12	<190	<24	0,0
2020-23-366-1	14,0	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	77,7
2020-23-367-1	13,8	121	<15	<52	<79	<13	<22	46	<10	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-368-1	15,0	123	<15	<52	<79	<13	<22	37	13	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-380-1	13,3	120	<15	<52	80	<13	<22	41	<10	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-381-1	14,0	71	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	0,0
BARLEY Region Midt																	
2020-23-303-1	10,9	219	38	<52	126	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	14,3
2020-23-303-3	8,3	316	82	<52	178	<13	<22	<30	65	<21	<55	<12	<39	<12	<190	<24	4,0
2020-23-305-1	9,3	130	16	<52	141	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	91,9
2020-23-306-1	13,0	<66	<15	<52	<79	<13	57	<30	<10	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-307-1	12,4	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	20,2
2020-23-369-1	10,3	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	1,4
2020-23-370-1	14,0	104	21	<52	<79	<13	<22	<30	14	<21	<55	<12	<39	<12	<190	<24	8,3
2020-23-372-1	15,4	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	0,0
2020-23-373-1	15,2	356	38	<52	<79	<13	<22	<30	69	<21	<55	35	<39	<12	<190	36	58,3
2020-23-373-2	14,6	515	47	<52	81	<13	<22	<30	36	<21	<55	<12	<39	<12	<190	<24	0,0
BARLEY Region Sør og Vest																	
2020-23-211-1	16,4	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	10,7
2020-23-298-1		151	<15	<52	<79	<13	<22	<30	<10	<21	<55	70	143	<12	<190	107	34,2
2020-23-301-1	17,9	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24	40,0
2020-23-301-3	15,3	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	37	<190	<24	1,1

Appendix Table 3: Results on mycotoxins in individual samples of complete feed for pigs (20 samples) 2020. 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=nivalenol, ZEN=zearealenone, OTA=ochratoxin A.

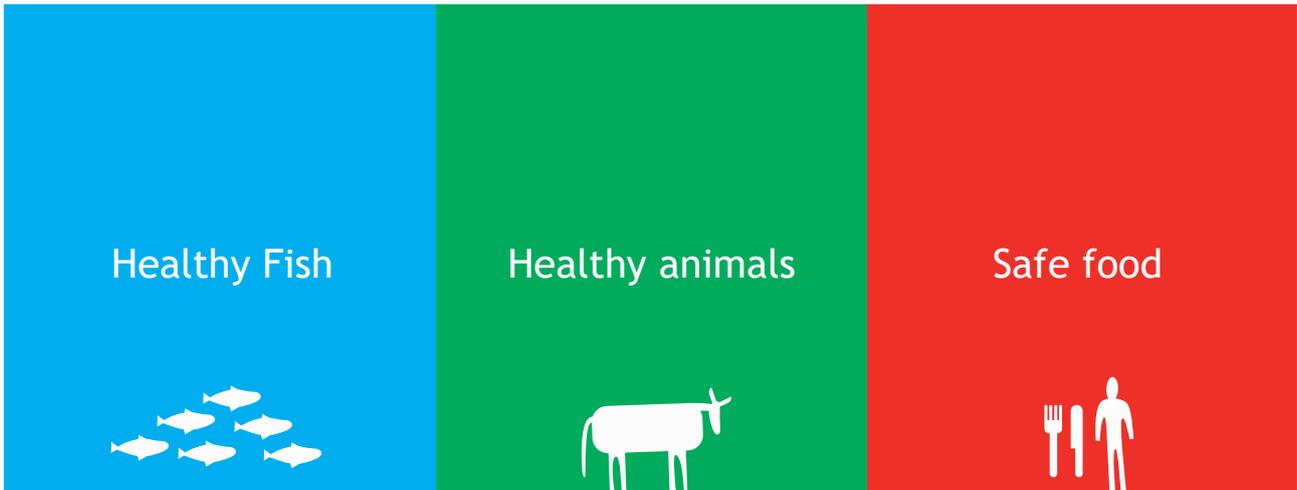
ID-nr.	Type of feed	DON	3-ac-DON	15-ac-DON	DON-3-G	T-2	HT-2	NIV	ZEN	OTA	Ergo-novine	Ergo-sine	Ergot-amine	Ergo-cornine	α-ergo-cryptine	Ergo-cristine
2020-21-14-1	NF Ideal Returnmelkkombi	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-19-1	Fiskå Opti Vital Pluss	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-32-1	Fullfor til svin	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-53-1	Fiskå Opti Norm	<66	<15	<52	<79	<13	23	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-57-1	Format Purke Soft	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-57-2	Format Kvikk 160 (F)	<66	<15	<52	<79	<13	<22	48	21	<21	<55	<12	<39	<12	<190	<24
2020-21-91-1	Fullfôr til svin	<66	<15	<52	<79	<13	39	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-107-1	Format Purke	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-108-1	Format Vekst 120	158	<15	<52	<79	<13	43	<30	10	<21	<55	59	<39	<12	<190	36
2020-21-109-1	Format Vekst 120	129	51	<52	<79	<13	22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-110-1	Format Laktasjon	77	<15	<52	<79	<13	<22	31	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-116-1	Fiskå Avlsfôr	199	<15	<52	<79	31	66	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-116-2	Fiskå Opti Vital Pluss	<66	<15	<52	<79	<13	<22	<30	10	<21	<55	<12	<39	<12	<190	<24
2020-21-120-1	Format Kvikk 140	84	<15	<52	<79	17	33	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-123-1	Ideal 70	<66	<15	<52	<79	<13	<22	43	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-130-1	Ideal 30	<66	<15	<52	<79	<13	34	<30	<10	<21	<55	48	<39	<12	<190	82
2020-21-130-2	Purkefor	69	<15	<52	<79	<13	<22	<30	<10	<21	<55	14	<39	<12	<190	<24
2020-21-133-1	Opti Appetitt	<66	<15	<52	<79	<13	<22	47	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-134-1	Format Purke	111	<15	<52	<79	<13	32	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-21-141-1	Format Vekst 105	194	28	<52	<79	<13	49	<30	17	<21	<55	<12	<39	<12	<190	<24

Appendix Table 4: Results on fungi (total mould, *Penicillium* spp., *Aspergillus* spp., Mucorales, yeasts; all cfu/g) and mycotoxins (all µg/kg) in nine individual samples of farm-mixed feed for ruminants in 2020. 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, ZEN=zearalenone, OTA=ochratoxin A.

ID-nr.	Total moulds	<i>Penicillium</i>	<i>Aspergillus</i>	Mucorales	Yeasts	DON	3-Ac-DON	15-Ac-DON	DON-3-G	T-2	HT-2	NIV	ZEN	OTA	Ergo-novine	Ergo-sine	Ergot-amine	Ergo-cornine	α-ergo-cryptine	Ergo-cristine
2020-23-239-1	45000	25000	15000	5000	55000000	<66	<15	<52	<79	<13	<22	<30	102	<21	<55	<12	<39	<12	<190	<24
2020-23-240-1	2800	2800	25	25	55000000	<66	<15	<52	<79	<13	35	<30	15	<21	<55	<12	<39	<12	<190	<24
2020-23-359-1	33000	33000	25	50	27000	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-360-1	25	25	25	25	25	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-361-1	50	50	25	25	83000	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-362-1	2500	300	25	2500	26000000	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-363-1	8200	8200	25	200	21000	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-379-1	50	25	25	25	50	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24
2020-23-379-2	50	50	25	25	25000000	<66	<15	<52	<79	<13	<22	<30	<10	<21	<55	<12	<39	<12	<190	<24

Appendix Table 5: Results on aflatoxin B1, B2, G1, G2 ($\mu\text{g}/\text{kg}$) in 10 individual samples of maize 2020

ID-nr.	Type	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2
2020-21-12-1	Whole maize	<0.25	<0.10	<0.20	<0.15
2020-21-13-1	Whole maize	<0.25	<0.10	<0.20	<0.15
2020-21-22-1	Whole maize	<0.25	<0.10	<0.20	<0.15
2020-21-23-1	Whole maize	<0.25	<0.10	<0.20	<0.15
2020-21-37-1	Maize gluten	<0.25	<0.10	<0.20	<0.15
2020-21-85-1	Maize gluten	5.31	0.5	<0.20	<0.15
2020-21-88-1	Whole maize	<0.25	<0.10	<0.20	<0.15
2020-21-89-1	Whole maize	<0.25	<0.10	<0.20	<0.15
2020-21-106-1	Maize gluten	<0.25	<0.10	<0.20	<0.15
2020-21-132-1	Maize gluten	<0.25	<0.10	<0.20	<0.15



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