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The surveillance programme for feed and feed materials in Norway 2019 - Mycotoxins, fungi and bacteria

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The surveillance programme for feed and feed materials in Norway 2019 – Mycotoxins, fungi and bacteria

Content

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Summary

The surveillance programme for feed and feed materials in 2019 included selected mycotoxins and fungi in oats, barley and farm-mixed feed for ruminants, aflatoxins in maize, selected mycotoxins in compound feed for pig, as well as selected bacteria in raw feed for dogs.

In oats were found higher total mould counts than guidance values in 60 % of the samples, and *Fusarium* levels characterised as high (above 25 000 cfu/g) in 52 % of the samples. Compared with the previous years, the *Fusarium* level was relatively high, but the levels of total moulds as well as of yeasts were more similar. The level of storage moulds was extraordinary low in 2019. The level of *Fusarium* in oats did not reflect levels of mycotoxins such as deoxynivalenol (DON) and zearalenone (ZEN) but some samples contained relatively high concentrations of T-2 and HT-2 toxin. For the first time, the levels of T-2 and HT-2 were present at the same level in oats samples from the Midt region as in the regions in South-Eastern Norway. Ochratoxin A (OTA) was not detected in oats.

Barley was examined for *Claviceps purpurea* (ergot) which was detected at low levels in 60 % of the samples – all far below the maximum limit. Ergot alkaloids were present in some barley samples – in one sample at rather high level of possible animal health concern. There was no significant correlation between ergot and ergot alkaloids. Trichothecenes and ZEN were detected only at low concentrations in a few samples and OTA was not detected in barley.

In maize, aflatoxins were detected below the maximum level in two samples of maize gluten, while none were detected in eight samples of whole maize.

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

Some samples of farm-mixed feed for ruminants contained high levels of storage mould or yeast above the guidance levels, while DON, T-2/HT-2, NIV and ZEN were absent or hardly present.

Salmonella bacteria were found in two samples of raw feed for dogs, both imported from England. *Clostridium perfringens* was detected in 31 % of the samples with one sample (1 %) at a level indicating poor hygienic quality and with potential of causing haemorrhagic enteritis. *Escherichia coli* was present in 69 % of the samples and in five samples (7 %) at elevated level indicating poor hygienic quality.

Sammendrag

I overvåkingsprogrammet for mykotoksiner og sopp i fôr og fôrråvarer i 2019 ble ulike mykotoksiner og sopp målt i havre, bygg og i gårdsblandet drøvtyggerfôr. Videre ble aflatoksiner målt i mais, og utvalgte mykotoksiner målt i kraftfôr til gris, og utvalgte bakterier i rått hundefôr.

I havre var 60 % av prøvene over veiledende grenseverdi for total muggsopp, og det var høyt innhold av *Fusarium* (over 25 000 kde/g) i 52 % av prøvene. Sammenlignet med tidligere år var *Fusarium*-nivået relativt høyt i 2019, mens total muggsopp og gjær ikke skilte seg spesielt ut. Lagringsmuggsopp i kornet var ekstraordinært lavt i 2019. Mye *Fusarium* gjenspeilet ikke nivåer av mykotoksiner som deoksynivalenol (DON) og zearalenon (ZEN), men noen havreprøver inneholdt relativt høye konsentrasjoner av T-2- og HT-2-toksin. For første gang var nivået av T-2 og HT-2 på samme nivå i region Midt som i regionene i Sørøst-Norge. Okratoksin (OTA) ble ikke påvist i havre.

I bygg ble det undersøkt for *Claviceps purpurea* (meldrøye) som ble påvist i 60 % av prøvene - alle langt under gjeldende grense. Meldrøye-alkaloider ble funnet i noen byggprøver – i en prøve i høyt nivå av mulig betydning for dyrehelsen. Det ble ikke påvist sammenheng mellom meldrøye og meldrøye-alkaloider. Trikotecener og ZEN ble kun påvist i lave konsentrasjoner i få prøver, og OTA ble ikke påvist i bygg.

I mais ble aflatoksiner påvist i to prøver av maisgluten i nivåer under grenseverdien, men resterende åtte prøver av hel mais var negative.

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

Enkelte prøver av gårdsblandet fôr til drøvtyggere inneholdt høye nivåer av lagringsmuggsopp eller gjærsopp over anbefalte grenser. DON, T-2/HT-2, NIV og ZEN ble ikke eller nesten ikke påvist i drøvtyggerfôret.

I det rå hundefôret ble *Salmonella*-bakterier funnet i to prøver som begge var importert fra England. *Clostridium perfringens* ble påvist i 31 % av prøvene av hundefôr, der én prøve (1 %) hadde et nivå av denne bakterien som indikerer redusert hygienisk kvalitet og potensiale for å kunne forårsake blodig tarmbetennelse. *Escherichia coli* ble påvist i 69 % av hundefôrprøvene, hvorav fem prøver (7 %) var over et nivå som indikerer redusert hygienisk kvalitet.

Introduction

The annual surveillance programme for mycotoxins and microorganisms in feed materials, and complete and complementary feed 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 advices from NVI, with NFSA responsible for collecting the samples, NVI for analysing and reporting of the results, and finally NFSA for result management.

Moulds can roughly be divided into field fungi 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 grains include species of *Fusarium*, *Alternaria*, *Microdocchium*, *Cladosporium*, *Acremonium*, *Epicoccum*, *Phoma* etc. Storage fungi usually occur in small amount before harvest. Under improper storage conditions, these small amounts can grow rapidly leading to significant problems. The most common storage fungi are the species of *Penicillium*, *Aspergillus* and Mucorales.

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 found DON to occur in high concentrations, particularly in oats and wheat. DON is hazardous to health if ingested by animals and humans [1]. Reduced feed intake and stunted growth rate in pigs caused by exposure to DON are well-documented gastrointestinal disorders. T-2 and HT-2 are usually present in levels of concern only in oats and oat products. T-2 and HT-2 have similar but potentially stronger toxic effects than DON, in causing gastrointestinal lesions as well as immune suppression [1]. Based on limited available occurrence 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 are produced by *Claviceps purpurea*, and this mould is found mainly in rye, but also occur in other cereal species. Barley seems to be more susceptible to *Claviceps purpurea* 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 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. The toxin may also suppress the immune response and growth performance [1]. As far as we know, OTA has not caused problems for Norwegian husbandry thus far, but active surveillance of OTA is important because of import of feed ingredients [1]. Aflatoxins, produced by some Aspergilli, are potential problems in feed import for Norway. To minimize human health risks via consumption of animal products, the carcinogenic and liver toxic aflatoxins in feed must remain at low levels. Aflatoxins in feed for dairy cattle can lead to an active aflatoxin metabolite in milk.

Raw feed for dogs are popular but there is currently little knowledge on the bacteriological/hygienic quality of such products with regards to *Salmonella*, *Clostridium perfringens* and *Escherichia coli*. *Salmonella* is an important pathogen and zoonotic bacteria. *Cl. perfringens* is an obligate anaerobic bacterium normally present in the colon of healthy animals. *E. coli* is commonly enumerated as an indication of faecal contamination and may give indications of the hygenic quality of the raw material and the production.

Aims

The aims of this programme on surveillance of feed and feed materials in Norway are to provide reliable documentation on the occurrence of important mycotoxins, selected fungi and contagious bacteria. 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 2019, the surveillance programme for feed consisted of the following samples shown in Table 1.

Table 1. Samples in the surveillance programme for feed 2019.

Matrix	Planned	Sampled and analysed	Analyses
Oats	45	42	Total moulds, *Fusarium*, storage moulds, yeasts, trichothecenes, zearalenone, ochratoxin A, ergot alkaloids
Barley	45	50	*Claviceps purpurea*, trichothecenes, zearalenone, ochratoxin A, ergot alkaloids
Maize/maize gluten	15	10	Aflatoxins
Complete compound feed for pigs	20	20	Trichothecenes, zearalenone, ochratoxin A, ergot alkaloids
Farm-mixed feed for ruminants	25	18	Total moulds, *Penicillium*, *Aspergillus*, yeasts, trichothecenes, zearalenone, ochratoxin A, ergot alkaloids
Raw feed for dogs	80	74	*Salmonella*, *Clostridium perfringens*, *Escherichia coli*

Samples of oat and barley from mills in grain production areas were sampled during autumn. Maize samples from imported batches from third countries, samples of compound feed for pigs from the feed industries and samples for farm mixed feed for ruminants were sampled throughout the year. Sampling followed EU Regulation 691/2013 to ensure samples were representative.

Quantitative determination of moulds and yeasts in oats and farm-mixed feed for ruminants

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).

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

Claviceps purpurea sclerotia in grams per kg cereal were calculated according to the recommendation of EFSA. 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 [5]. 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 were demonstrated for all selected mycotoxins, varying from 27 % to 96 %. 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) 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 x SD/m* (Table 2).

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

Toxin	LOD, µg/kg	Total within laboratory precision (%)				Recovery ± SD (%)			
		Oats	Barley	Pig feed	Ruminant feed	Oats	Barley	Pig feed	Ruminant feed
Deoxynivalenol	66	8	5	4	9	127±2	127±6	119±7	117±17
DON-3-glucoside	79	8	7	31	5	116±14	118±3	97±28	101±4
3-Acetyl-DON	15	8	4	20	6	131±11	121±6	143±33	109±1
15-Acetyl-DON	52	3	5	8	6	134±7	135±7	178±14	170±6
Nivalenol	30	7	6	26	10	131±5	130±10	99±30	109±13
T-2 toxin	13	5	3	40	9	125±5	122±1	177±68	122±12
HT-2 toxin	22	5	5	25	5	128±4	120±9	167±45	123±10
Zearalenone	10	3	4	43	3	109±7	122±3	184±78	116±3
Ochratoxin A	21	9	5	36	7	105±15	113±9	146±52	104±12
Ergonovine*	55	6	6	12	7	210±15	125±10	139±16	96±4
Ergosine*	13	6	8	10	9	155±9	126±9	144±17	77±7
Ergotamine*	39	5	6	25	8	135±9	112±9	142±33	67±9
Ergocornine*	12	3	8	23	3	152±2	119±13	168±40	80±6
α-Ergocryptine*	185	7	11	34	3	140±10	107±8	161±54	86±2
Ergocristine*	24	8	5	37	3	127±11	110±10	180±67	79±1

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

The extraction methodology was based on the two-step extraction (MeCN:H_2O: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 immunoaffinity columns clean up followed by determination by HPLC using fluorescence detection after post-column derivatisation. The LOD for aflatoxins were: B1: 0.25 µg/kg, B2: 0.10 µg/kg, G1: 0.20 µg/kg, G2: 0.15 µg/kg.

Bacterial analysis of feed for dogs

The samples of frozen raw feed for dogs received by the laboratory were kept in the freezer (<16°C) until analysis. All samples were analysed within their use-by-date. The samples were analysed quantitatively for *E. coli* and *C. perfringens* using 3M Petrifilm ™ Select *E. coli* Count Plate (3M Health Care St. Paul, MN, USA) and NMKL no. 95, 5th ed, 2009, respectively. *Salmonella* was detected by using NMKL no. 187, 2nd ed, 2016.

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. Bar graphs with standard errors and linear regression analysis were used to determine whether the combined T-2+HT-2 levels in oats varied geographically over time (2016-2019). Half the detection limits specific to a variable were used for calculation purposes if levels were not detectable.

Results and discussion

Cereals

Fungi and mycotoxins in oats

In oats were measured total moulds, *Fusarium* spp., storage moulds, and yeasts. Total mould counts, detectable in all samples, were above guidance value (500,000 cfu/g) [6], indicating poor hygienic quality, in 60 % of the samples (Table 3). In general, feed with reduced hygienic quality can cause reduced growth and health problems in animals [7]. However, fresh grains from the field may naturally contain high levels of field fungi, which are eliminated during common drying. Thus, not yet sufficiently dried grains, should be emphasized milder than stored grains.

Fusarium and yeasts were detected in all samples of oats, while storage moulds were found in one third of the samples. *Fusarium* levels above 25,000 cfu/g, considered potentially hazardous, were found in 52 % of the samples. No cereal samples exceeded the guidance value for yeasts (10,000,000 cfu/g) [6], and no samples exceeded the guidance value for storage moulds (100,000 cfu/g) [6].

The levels of total moulds and yeasts in 2019 for oats varied little from 2018 [8]. However the *Fusarium* level for oats in 2019 were higher, with mean and median about 3 times higher in 2019 compared with 2018, and also somewhat higher than in 2017 but similar as in 2016 [3,4]. The level of storage moulds was extraordinarily low in 2019.

Table 3. Occurrence of fungi (cfu/g of total moulds, *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), 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 2019.

	Total moulds	*Fusarium* spp.	Storage moulds	Yeasts	DON	3-Ac-DON	15-Ac-DON	DON -3-G	T-2+ HT-2	NIV	ZEN
Mean	885 000	31 500	800	618 500	166	29	<52	<80	292	56	17
Median	500 000	28 500	<50	500 000	<66	<16	<52	<80	104	<30	<10
Minimum	34 000	500	<50	5 000	<66	<16	<52	<80	<36	<30	<10
Maximum	4 400 000	140 000	15 000	2 400 000	2 146	269	156	312	2 410	408	286
SD*	847 400	27 400	2 800	525 800	357	45	20	60	561	83	46
% samples >dl*	100	100	290	100	45	50	2	12	83	40	14
% samples >gv*	60	52	0	0	0				10		0

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

The levels of total moulds and *Fusarium* in oats were highly positively correlated (p<0.0001) (Table 4). Furthermore, total moulds were also significantly correlated with yeast level (p=0.03), whereas storage moulds were not correlated with other fungi.

Table 4. Correlation coefficients between counts of the various groups of fungi in oats (N = 42) sampled in Norway in 2019. Significant correlation coefficients are presented in bold (p<0.05).

	Total moulds	*Fusarium* spp.	Storage moulds	Yeasts
Total moulds	1.000			
Fusarium spp.	**0.722**	1.000		
Storage moulds	-0.144	-0.171	1.000	
Yeasts	**0.327**	0.148	0.060	1.000

Table 5 shows the frequency of the dominating *Fusarium* species found in oats. *Fusarium poae* was the dominating species in 2019 similar to 2018, and *F. langsethiae* and *F. graminearum* were also similarly present these two years. However, the detection of *F. avenaceum* increased greatly in frequency in 2019 compared to 2018. *F. poae* may produce NIV and ZEN and other secondary fungal metabolites but is not reported to produce DON [9]. *F. langsethiae* and *F. graminearum* are the main producers of T-2/HT-2 and DON, respectively, whereas *F.avenaceum* does not produce trichothecenes, but less important mycotoxins such as moniliformin, enniatins and others [1].

Table 5. The frequency of the dominating *Fusarium* species found in oats (N = 42) sampled in Norway in 2019.

Species	Number (%) of samples detected	Number of samples with most dominant species
F. poae	36 (86 %)	23
F. avenaceum	24 (57 %)	8
F. langsethiae	9 (21 %)	3
F. graminearum	6 (14 %)	4
F. tricinctum	4 (10 %)	0
F. culmorum	1 (2 %)	0
Fusarium sp. (not identified)	1 (2 %)	

In 2019, DON and DON-related compounds in oats were found at very low levels similarly as in 2017 and 2018. No oat samples exceeded the limit for DON recommended by EU and Norway (8000 µg/kg) [6, 10].

The DON-related compounds included in the analysis 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 levels in half of the samples, whereas DON-3-G was found in fewer samples. 15-Ac-DON was only detectable in one sample. DON and 3-Ac-DON were significantly positively correlated in oats (Figure 1). In general, the related compounds contribute substantially to the total DON concentration [11].

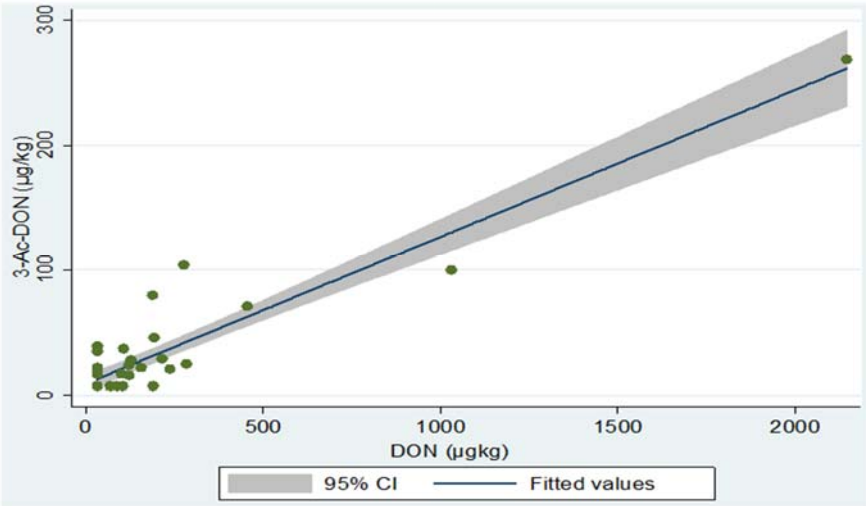

Figure 1. The Pearson correlation (r) between deoxynivalenol (DON) and 3-acetyl-DON in oats (N=42) were 0.930, p<0.0001. A regression line with 95 % confidence interval fitted to the points allows predictions of levels of 3-Ac-DON given the level of DON detected and vice versa.

T-2 and HT-2 for which recommendations exist as the sum of T-2 + HT-2 in EU and Norway [6,12] showed four samples with concentrations above guidance level of 500 µg/kg (Table 3; Figure 2). Thus, their mean concentration was somewhat higher in 2019 compared with the last years [3, 8]. HT-2 and T-2 were highly correlated in oats (Figure 2).

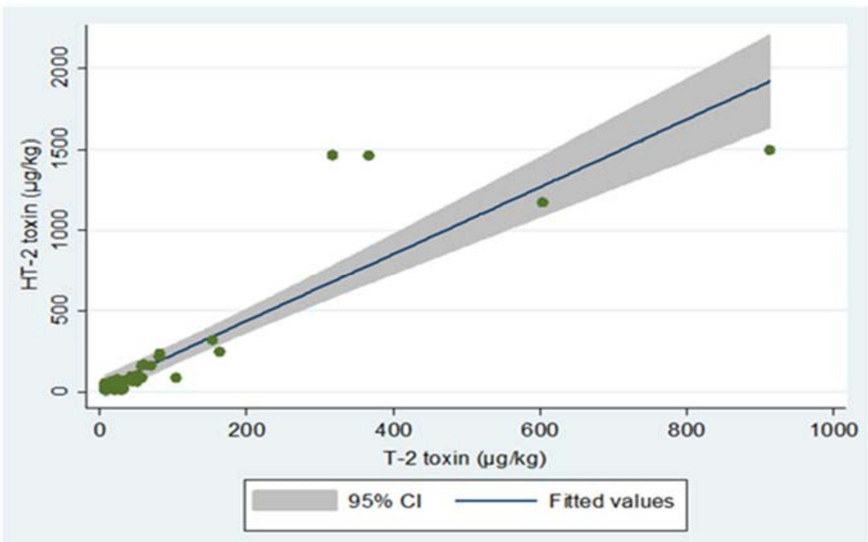

Figure 2. The Pearson correlation (r) between T-2 toxin and HT-2 toxin in oats (N=42) were 0.893 (p<0.0001). A regression line with 95 % confidence interval fitted to the points allows predictions of levels of HT-2 toxin given the level of T-2 toxin detected and vice versa.

The concentrations of DON and T-2 + HT-2 in oats have been determined in surveillance programs since 2002 and Figure 3 illustrates that mean DON concentration in 2019 was similar as the last previous years – at the lower end of the scale. In fact, the DON concentrations have been relatively low the last years since the peak DON level in 2012. Figure 3 also shows that mean concentration of T-2+HT-2 in 2019 was somewhat higher than the three last years, more similar as in 2014 and 2015.

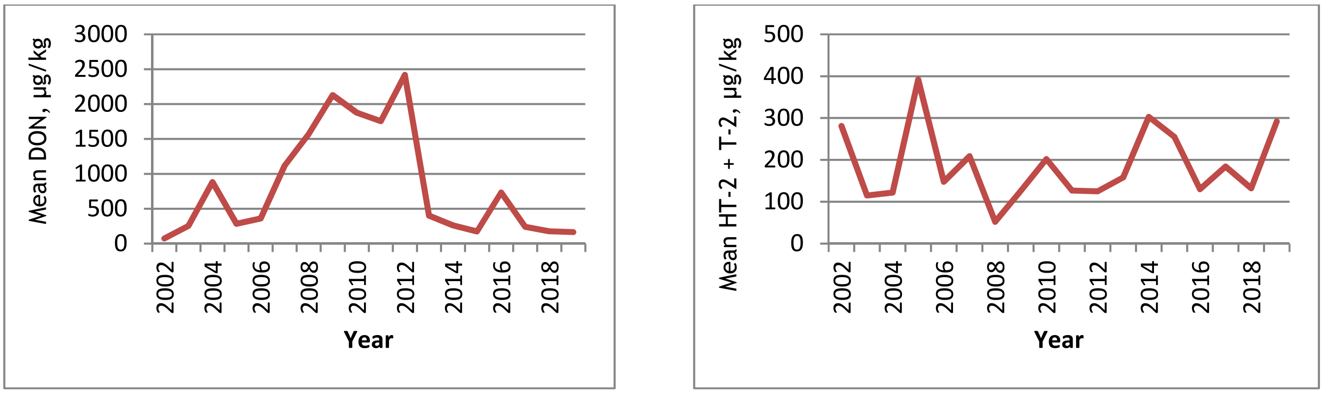

Figure 3. Mean concentration of deoxynivalenol (DON) (left) and the sum of T-2 toxin and HT-2 toxin (right) in 30-60 samples of oats per year in the Norwegian surveillance programme for feed.

NIV was only detected in a few oat samples (Table 3), which is as usual in Norway. Also ZEN was only detected in a few samples, mostly at trace amounts, except one sample at 286 µg/kg, but still far below the recommended limit (2000 µg/kg) in EU and Norway (Table 3) [6,10]. OTA was not detected in oats.

Even though ergot alkaloids surveillance in oats was not ordered by the Norwegian Food Safety Authority, these compounds were analysed by the same method together with the other mycotoxins and their results were included in the report. Except ergosine detected at trace amount 20 µg/kg in one sample, no ergot alkaloids were found. Insignificant or not detectable levels of ergot alkaloids are common finding 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]. In 2019, the temperature was somewhat above the normal for July, particularly in Trøndelag (region Midt), and the precipitation was below the normal [13]. Also August was relatively warm, with somewhat more precipitation than normal both in August and September. The pattern of moulds and mycotoxins in 2019 showed relatively high level of *Fusarium*, low levels of storage moulds, and more T-2/HT-2 compared with the year before. The results correspond well with the meteorological data.

Regional differences were analysed for fungi and mycotoxins in oats. Less total moulds and less *Fusarium*, were found in region Midt (Trøndelag and Møre/Romsdal) than in the regions in South-Eastern Norway (Table 6). Regarding mycotoxins, none showed significant regional differences. Of particular interest is that the concentrations of T-2+HT-2 had similar level in regions of Midt and South-Eastern Norway (Table 6). The occurrence of T-2 and HT-2 in the middle of Norway has increased during the last years. Increasingly warmer climate over the years is a probable explanation. Several studies indicate that a relatively warm and dry climate is necessary for the production of T-2 and HT-2 [14,15]. In 2016 and the years before, these toxins were hardly found in oats from region Midt. In 2017 and 2018, T-2+HT-2 became more present in samples from this region, but at lower level than in other regions.

Figure 4 shows the mean and standard error margins for T-2+HT-2 in oats from region Midt compared with those from South-Eastern Norway (combined data for regions Stor-Oslo and Øst) during the years 2016-19. The responsible producer of T-2/HT-2, *Fusarium langsethiae,* has for many years been documented in Midt at a similar level as further south [16]. With warmer growing seasons in Midt region as observed in 2017, 2018 and 2019 the levels of T-2 and HT-2 in this region now seem to have reached similar levels as in South-Eastern Norway. However, there is uncertainty to the results due to low number of samples from region Midt.

Table 6. Survey between regions Øst (counties Buskerud, Vestfold, Telemark, Hedmark, Oppland), Stor-Oslo (Akershus, Oslo, Østfold) and Midt (Trøndelag and Møre/Romsdal) on fungi (total mould, *Fusarium* spp., storage mould and yeast (cfu/g) and trichothecenes (deoxynivalenol (DON), sum of T-2 and HT-2 toxin, nivalenol) and zearalenone (all toxin concentrations µg/kg) in oats (N = 42) sampled in Norway in 2019. Variables that were significantly different between regions are indicated by an * (p<0.05).

Region		Total moulds*	*Fusarium* spp.*	Storage moulds	Yeasts	DON	T-2+ HT-2	NIV	ZEN
Øst n=15	Mean	1 081 000	47 700	97	739 700	291	413	87	14
	St. dev.	1 071 800	29 200	170	597 500	572	691	124	24
Stor-Oslo n=20	Mean	990 000	27 000	940	609 000	115	208	47	23
	St. dev.	680 800	22 000	2430	520 500	113	391	44	63
Midt n=7	Mean	165 000	9 790	2 180	385 700	<66	274	<30	<10
	St. dev.	150 200	18 000	5 650	313 500	34	637	0	0

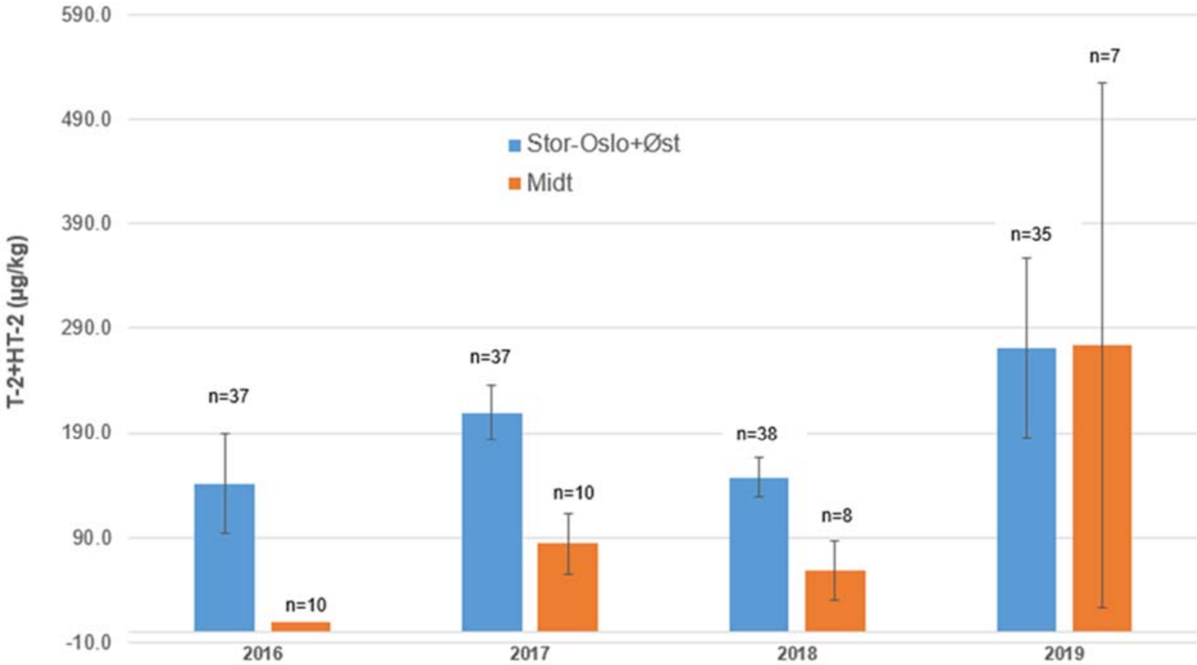

Figure 4. Concentrations (mean and standard error of the mean) of T-2+HT-2 toxin in oats from region Midt and regions Stor-Oslo + Øst during the years 2016-19.

Fungi and mycotoxins in barley

Total moulds, total *Fusarium*, yeasts and storage moulds were exempted from surveillance by the Norwegian Food Safety Authority for barley in 2019. On the other hand ergot, *Claviceps purpurea,* were included in the analysis repertoire of 2019. *C. purpurea* was detected in 60 % of the barley samples, with an overall mean level of 7 mg/kg and a maximum of 73 mg/kg (Table 7). Thus, all samples were far below the maximum concentration of 1000 mg/kg [6]. Most of the ergot sclerotia were small in size which indicates that they had been growing on grass. Only four samples had sclerotia in the same size as, or bigger than, a barley grain/kernel, indicating that the ergot had grown on the barley. Ergot alkaloids were present in several of the samples, particularly ergosine and ergocristine and partly ergocornine. The maximum concentrations of ergosine and ergocristine were 1040 and 1760 µg/kg, respectively, found in the same sample (Table 7). Considerably high levels were only sporadically present, but the results verifies that ergot alkaloids may occur in elevated levels in barley. From 2016, ergot alkaloids were included in the analysis repertoire of barley, showing that ergot alkaloids were found at similar maximum level in 2016 and 2017 [3,4]. However, in 2019 the number of samples with detectable levels of these alkaloids were increased, and the results indicate a higher incidence of ergot alkaloids in barley from regions of Midt and Sør-Vest than in South-Eastern Norway (Appendix Table 2).

Table 7. 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 = 50) sampled in Norway in 2019.

	C. purpurea sclerotia	Ergo-novine/ -inine	Ergo-sine/-inine	Ergot-amine/-inine	Ergo-cornine/-inine	α-Ergo-cryptine/-inine	Ergo-cristine /-inine	∑ Ergot alkaloids
Mean	7	<56	45	<40	<12	<190	70	<332
Median	3	<56	<12	<40	<12	<190	<24	<332
Minimum	0	<56	<12	<40	<12	<190	<24	<332
Maximum	73	<56	1 043	<40	21	<190	1 764	2 971
SD*	0	0	163	0	2	0	257	422
% samples >dl*	60	0	20	0	4	0	14	4

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

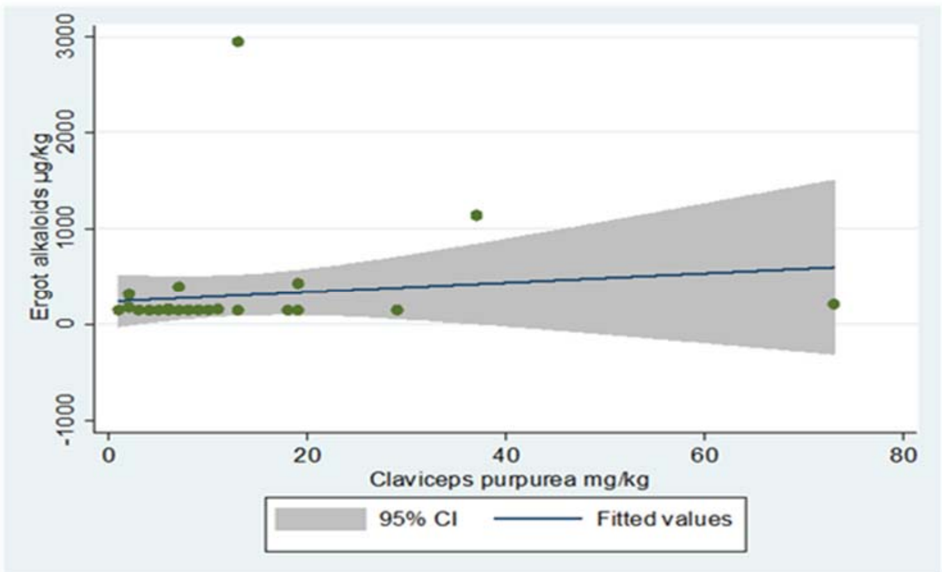

Figure 5. The Pearson correlation (r) between *Claviceps purpurea* and sum of ergot alkaloids detected (ergosine, ergocristine, ergocornine) in samples of barley (n = 50) were 0.194 (p=0.170). A regression line fitted to the points with 95 % confidence interval is shown.

No significant correlation were found between the ergot and the ergot alkaloids in the barley (Figure 5). Lack of correlations between ergot and alkaloids were also found in previous surveys in Norway, in studies of rye and wheat in 2016 and 2017 [17,18]. These results indicate variable production of alkaloids by the ergot fungi. The legislative control of *C. purpurea* sclerotia is thus of little value in toxicological risk management concerning ergot alkaloids.

Even though data on toxic effects of ergot alkaloids for risk assessment are sparse, the maximum levels found here could be of possible animal health concern if using barley from the present batch as a major feed ingredient [3].

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

Table 8. Occurrence (µg/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 barley (n = 50) sampled in Norway in 2019.

	DON	3-Ac-DON	15-Ac-DON	DON-3-G	T-2+ HT-2	NIV	ZEN
Mean	<66	<16	<52	<80	<36	<30	<10
Median	<66	<16	<52	<80	<36	<30	<10
Minimum	<66	<16	<52	<80	<36	<30	<10
Maximum	451	32	<52	330	132	37	28
SD*	72	4	0	49	22	3	6
% samples >dl*	8	4	0	4	8	2	10
% 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 ten analysed maize samples, with highest concentrations of aflatoxin B1 and B2 detected at 15.3 and 2.0 µg/kg, respectively. No samples exceeded the maximum limit of aflatoxin B1 (20 µg/kg) [19]. The positive samples were maize gluten, whereas the negative samples were whole maize.

Feed

Feed for pigs

The levels of mycotoxins in complete compound feed for pigs in Table 9 show that DON was detected in two samples only (10 %), both below the recommended limit for feed for pig in Norway (500 µg/kg) [6]. Co-occurrence of DON-related compounds were mostly undetectable, only trace amounts of 3-Ac-DON were found 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 [11].

The sum of T-2 and HT-2 was present in two samples of the pig feed samples, but it did not exceed the recommended limit (250 µg/kg) [6,12]. The levels of DON and T-2/HT-2 were similar as in 2018 and lower than in 2016 and 2017 [3,4,8].

ZEN was present at low levels in three samples, all below the recommended level for pig feed in Norway (250 µg/kg) [6]. These results are unchanged the last years [3,4,8].

NIV and OTA were not detected in feed for pigs.

Ergot alkaloids were found at low level in one sample. That was ergosine and ergocristine at 122 and 393 µg/kg, respectively. Ergot alkaloids were not detected in pig feed in 2018, but were found in 2017 in a single sample at a total alkaloid concentration 1620 µg/kg [3,8].

Table 9. Concentrations of deoxynivalenol (DON), 3-acetyl-DON, 15-acetyl-DON, DON-3-glucoside, sum of T-2 and HT-2 toxin, nivalenol (NIV), zearalenone (ZEN) and sum of ergot alkaloids (µg/kg) in complete compound feed for pigs (N = 20) sampled in Norway in 2019.

	DON	3-Ac-DON	15-Ac-DON	DON-3-G	T-2+ HT-2	NIV	ZEN	∑ Ergot alkaloids
Mean	<66	<15	<52	<80	<36	<30	<10	< 330
Median	<66	<15	<52	<80	<36	<30	<10	< 330
Minimum	<66	<15	<52	<80	<36	<30	<10	< 330
Maximum	188	17	<52	<80	80	<30	38	664
SD*	37	3	0	0	18	0	7	111
% samples >dl*	10	10	0	0	10	0	15	5
% 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 (µg/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 = 18) sampled in Norway in 2019.

	Total moulds**	*Penicill-ium***	*Asper-gillus***	Yeasts**	DON	3-Ac-DON	15-Ac-DON	DON-3-G	T-2+ HT-2	NIV	ZEN
Mean	78 900	77 600	300	13 822 000	<66	<16	<52	<80	20	<30	<10
Median	9 100	500	25	530 000	<66	<16	<52	<80	<36	<30	<10
Minimum	<50	<50	<50	20 000	<66	<16	<52	<80	<36	<30	<10
Maximum	1 100 000	1 100 000	4 500	130 000 000	<66	<16	<52	<80	47	<30	10
SD*	264 700	265 000	1 100	37 200 000	0	0	0	0	7	0	1
% samples >dl*	88	82	18	100	0	0	0	0	6	0	6
% samples >gv*		12	0	12	0				0		0

* SD = Standard Deviation, >dl = above detection limits, >gv = above guidance values.
** Number of samples analysed (N) = 17, not 18.

Most of the growth consisted of storage moulds in the genera *Penicillium*, *Aspergillus* and *Mucor*. *Penicillium* spp. were found in 14 samples (82 %), and two of these were above guidance value (50,000 cfu/g) [6], indicating poor hygienic quality (Table 10). In both samples the mycoflora was dominated by *Penicillium* spp,, mainly the species *P. roqueforti*. In addition, in five samples (29 %) the occurrence of *Penicillium* spp. were between 10,000 – 50,000 cfu/g indicating reduced hygienic quality [6].

Aspergillus spp. were found above detection limit in three samples (18 %). None of these were above the guidance value (50,000 cfu/g).

Yeasts were found in all samples, and two samples (12 %) were above guidance value (10,000,000 cfu/g) indicating poor hygienic quality. In addition, in three samples (18 %) the occurrence of yeasts were between 1,000,000 – 10,000,000 cfu/g) indicating reduced hygienic quality.

Concerning mycotoxins, trichothecenes and ZEN were hardly detected, and OTA and ergot alkaloids were not detectable.

Feed for dogs

Raw, frozen by-products from slaughterhouses produced as feed for dogs were analysed for *Salmonella* sp., *Clostridium perfringens* and *Escherichia coli* (Table 11). *Salmonella* bacteria (*S. Derby* or *S. Typhimurium*) were isolated from two samples (both imported from England). *C. perfringens* was detectable in 23 of the 74 samples, at levels up to 1400 cfu/g. One sample had *C. perfringens* level above 1000 cfu/g indicating poor hygienic quality. Elevated levels of *C. perfringens* has the potential of causing haemorrhagic enteritis in dogs. *E. coli* was detectable in 51 samples. Five samples had *E. coli* exceeding 1000 cfu/g up to a maximum of 8800 cfu/g. *E. coli* may usually occur in raw slaughter by-products, however, the presence of *E. coli* is used as an indicator of faecal contamination, and high numbers of this bacteria indicates poor production hygiene because of faecally contaminated raw materials. A level of *E. coli* above 1000 cfu/g indicate poor hygienic quality. Occurrence of *Salmonella* or elevated numbers of *C. perfringens* or *E.coli* were not correlated and found in separate samples.

Raw, frozen slaughterhouse by-products meant for dogs were also examined in 2016 for the same bacteria with rather similar results [4]. In 2016 S*almonella* (*S. Mbandaka*) was found in one sample (imported from England), *C. perfringens* in 16 % of the samples and up to 1000 cfu/g, and *E. coli* in 87 % of the samples up to 70,000 cfu/g.

Table 11. Occurrence of *Clostridium perfringens* and *Escherichia coli* (cfu/g) in raw feed for dogs (N = 74) sampled in Norway in 2019.

	C. perfringens	*E. coli*
Mean	32	407
Median	<10	40
Minimum	<10	<10
Maximum	1 400	8 800
SD*	162	1 249
% samples >dl*	31	69
% samples >gv*	1	7

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

Conclusions

Feed materials

- **Fungi in oats**: Higher total mould counts than the guidance values were found in 60 % of the samples. *Fusarium* at potentially health concern level was found in 52 % of the samples. The *Fusarium* level (dominated by *F. poae* and *F. avenaceum*) was considered relatively high in 2019, but the levels of total moulds as well as of yeasts were about the same level as in 2018. The level of storage moulds was extraordinarily low in 2019.
- **Mycotoxins in oats**: The high level of *Fusarium* spp. did not reflect a correspondingly high level of mycotoxins such as deoxynivalenol and zearalenone. Some samples contained relatively high concentrations of T-2 and HT-2 toxin (four samples above the guidance level). Regional difference in levels of T-2 and HT-2 in oats samples disappeared in 2019 for the first time. Previously lower T-2/HT-2 levels were shown in oats from region Midt than in regions in South-Eastern Norway. Ochratoxin A was not detected.
- **Fungi in barley**: *Claviceps purpurea* (ergot) was detected in 60 % of the samples but at levels far below the maximum limit at 1000 mg/kg.
- **Mycotoxins in barley**: Ergot alkaloids were present in some samples – One sample had levels high enough for possible animal health concern. No significant correlation was found between ergot and ergot alkaloids. Trichothecenes and zearalenone were only detected at low concentrations in a few samples and ochratoxin A was not detected.
- **Aflatoxins in maize:** Aflatoxins were detectable in two out of ten samples - both samples were maize gluten. The maximum concentration of aflatoxin B1 was 15.3 µg/kg and no samples exceeded the maximum limit (20 µg/kg).

Feed

- **Compound feed for pig**: The mycotoxins deoxynivalenol, T-2/HT-2 toxin, nivalenol, zearalenone and ergot alkaloids were found at low levels in a few samples. Ochratoxin A was not detected.
- **Farm-mixed feed for ruminants**: Some of the samples contained high levels of storage mould or yeast above the guidance levels. Deoxynivalenol, T-2/HT-2 toxin, nivalenol and zearalenone were undetectable ore negligible.
- **Raw feed for dogs:** *Salmonella* bacteria (*S.* Derby and *S.* Typhimurium) were found in two frozen samples, both imported from England. *Clostridium perfringens* was detectable in 31 % of the samples with one sample (1 %) above 1000 cfu/g indicating poor hygienic quality and with the potential of causing haemorrhagic enteritis. *Escherichia coli* was present in 69 % of the samples and five samples (7 %) above 1000 cfu/g indicating poor hygienic standard in the feed production.

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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 2019. 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.

ID-nr.	Total moulds	*Fusarium*	Storage moulds	Yeasts	DON	3-Ac DON	15-Ac Don	DON-3-G	HT-2 + T-2	NIV	ZEN	OTA	Ergo-novine	Ergo-sine	Ergot-amine	Ergo-cornine	α-Ergo-cryptine	Ergo-cristine
OATS Region Øst																		
2019-23-210-3	590000	40000	<50	600000	<66	<16	<52	<80	62	330	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-212-2	1200000	59000	<50	1000000	69	<16	<52	<80	218	83	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-213-2	4400000	140000	<50	770000	<66	<16	<52	<80	1784	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-214-2	1100000	23000	<50	2400000	128	28	<52	<80	<36	76	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-215-2	500000	50000	<50	1100000	284	25	<52	100	230	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-216-2	550000	36000	<50	750000	1031	100	<52	308	112	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-274-2	640000	50000	500	650000	216	29	<52	<80	144	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-274-4	450000	50000	<50	140000	<66	<16	<52	<80	227	172	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-274-5	340000	27000	<50	70000	<66	<16	<52	<80	72	83	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-274-6	1800000	55000	500	1100000	<66	<16	<52	<80	52	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-303-1	270000	36000	<50	5000	<66	<16	<52	<80	83	<30	54	<21	<56	<12	<40	<12	<190	<24
2019-23-303-2	2100000	50000	<50	1100000	2146	269	156	312	2411	408	13	<21	<56	<12	<40	<12	<190	<24
2019-23-303-5	1100000	45000	100	350000	157	22	<52	<80	410	30	86	<21	<56	<12	<40	<12	<190	<24
2019-23-305-2	1000000	50000	<50	820000	108	37	<52	<80	317	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-305-3	170000	5000	<50	240000	<66	17	<52	<80	53	<30	<10	<21	<56	<12	<40	<12	<190	<24
OATS Region Stor-Oslo																		
2019-23-193-2	1300000	35000	5000	2100000	105	<16	<52	<80	130	83	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-199-2	1000000	30000	<50	820000	189	80	<52	<80	156	106	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-200-2	800000	18000	<50	400000	<66	39	<52	<80	189	66	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-204-2	290000	7000	<50	200000	<66	22	<52	<80	108	58	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-205-2	410000	30000	<50	500000	238	21	<52	<80	1830	137	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-206-2	1300000	20000	<50	640000	<66	35	<52	<80	67	43	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-222-2	910000	30000	150	450000	<66	<16	<52	<80	32	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-223-2	910000	50000	1700	550000	<66	<16	<52	<80	40	33	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-277-2	500000	15000	<50	450000	<66	<16	<52	<80	100	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-282-2	770000	5000	1000	600000	191	<16	<52	<80	114	54	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-283-2	640000	15000	<50	350000	102	17	<52	<80	298	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-284-2	1000000	5000	25	150000	<66	17	<52	<80	141	<30	<10	<21	<56	<12	<40	<12	<190	<24

2019-23-293-2	2000000	100000	500	350000	88	<16	<52	<80	80	<30	64	<21	<56	<12	<40	<12	<190	<24
2019-23-294-2	2400000	20000	<50	500000	122	24	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-345-2	2700000	45000	<50	910000	194	46	<52	<80	114	49	286	<21	<56	<12	<40	<12	<190	<24
2019-23-346-2	1100000	20000	<50	1900000	<66	<16	<52	<80	35	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-348-2	320000	15000	10000	640000	<66	21	<52	<80	72	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-353-2	700000	50000	<50	350000	276	104	<52	<80	473	<30	30	<21	<56	<12	<40	<12	<190	<24
2019-23-353-3	590000	20000	<50	250000	456	71	<52	120	128	164	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-354-2	160000	9000	50	70000	<66	<16	<52	<80	30	<30	<10	<21	<56	<12	<40	<12	<190	<24
OATS Region Midt																		
2019-23-218-2	48000	500	<50	200000	<66	<16	<52	<80	55	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-219-2	85000	4000	150	600000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-225-2	150000	2000	<50	1000000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-227-2	470000	50000	<50	170000	<66	<16	<52	<80	1778	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-342-1	140000	1500	15000	360000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-342-3	34000	500	<50	230000	123	16	<52	<80	<36	<30	<10	<21	<56	20	<40	<12	<190	<24
2019-23-343-2	230000	10000	<50	140000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24

Appendix Table 2. Results on mycotoxins (µg/kg) and *Claviceps purpurea* (mg/kg) in **barley** based on 50 individual samples from different regions 2019. 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.

ID-nr.	DON	3-Ac DON	15-Ac Don	DON-3-G	HT-2 + T-2	NIV	ZEN	OTA	Ergo-novine	Ergo-sine	Ergot-amine	Ergo-cornine	α-Ergo-cryptine	Ergo-cristine	*C. purpurea* sclerotia
BARLEY Region Øst															
2019-23-209-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	9
2019-23-210-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	8
2019-23-210-2	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
2019-23-212-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	10
2019-23-213-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	7
2019-23-214-1	<66	<16	<52	<80	104	<30	<10	<21	<56	<12	<40	<12	<190	<24	29
2019-23-215-1	451	32	<52	238	<36	<30	22	<21	<56	<12	<40	<12	<190	<24	0
2019-23-216-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	18
2019-23-274-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
2019-23-274-3	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	7
2019-23-303-3	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	4
2019-23-303-4	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
2019-23-305-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0

BARLEY Region Stor-Oslo														
2019-23-193-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	18
2019-23-199-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	1
2019-23-200-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
2019-23-204-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
2019-23-205-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
2019-23-206-1	<66	<16	<52	<80	<36	37	<10	<21	<56	23	<40	<12	<190	<24	6
2019-23-222-1	67	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	8
2019-23-223-1	<66	<16	<52	<80	<36	<30	27	<21	<56	<12	<40	<12	<190	<24	0
2019-23-277-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	6
2019-23-282-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	3
2019-23-283-1	<66	<16	<52	<80	81	<30	<10	<21	<56	<12	<40	<12	<190	<24	7
2019-23-284-1	320	19	<52	330	<36	<30	21	<21	<56	17	<40	<12	<190	64	73
2019-23-291-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	18
2019-23-293-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	5
2019-23-294-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	7
2019-23-345-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	19
2019-23-346-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
2019-23-347-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	3
2019-23-348-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
2019-23-353-1	<66	<16	<52	<80	<36	<30	23	<21	<56	<12	<40	<12	<190	<24	0
2019-23-354-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
BARLEY Region Midt														
2019-23-218-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
2019-23-218-3	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
2019-23-219-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
2019-23-220-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	1
2019-23-225-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	57	<40	<12	<190	155	0
2019-23-225-3	<66	<16	<52	<80	<36	<30	<10	<21	<56	37	<40	<12	<190	<24	2
2019-23-226-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
2019-23-227-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
2019-23-342-2	<66	<16	<52	<80	132	<30	<10	<21	<56	1043	<40	21	<190	1764	13
2019-23-343-1	135	<16	<52	<80	45	<30	28	<21	<56	48	<40	<12	<190	139	2
2019-23-344-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	0
BARLEY Region Sør-Vest														
2019-23-198-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24	13

2019-23-198-2	<66	<16	<52	<80	<36	<30	<10	<21	<56	519	<40	<12	<190	494	37
2019-23-207-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	164	<40	14	<190	127	19
2019-23-207-2	<66	<16	<52	<80	<36	<30	<10	<21	<56	19	<40	<12	<190	<24	11
2019-23-233-1	<66	<16	<52	<80	<36	<30	<10	<21	<56	60	<40	<12	<190	206	7

Appendix Table 3. Results on mycotoxins in individual samples of **complete feed for pigs** (20 samples) 2019. 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=zearalenone, 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
2019-21-3-1	Ideal S Die	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-4-1	Opti Appetitt	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-6-1	Format Vekst 110	188	<16	<52	<80	72	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-8-1	Opti Norm	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-14-1	Complete pig feed	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-14-2	Complete pig feed	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-14-3	Complete pig feed	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-14-4	Complete pig feed	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-16-1	Format Laktasjon	99	<16	<52	<80	<36	<30	<10	<21	<56	122	<40	<12	<190	393
2019-21-19-1	Format Drektig	<66	16	<52	<80	80	<30	10	<21	<56	<12	<40	<12	<190	<24
2019-21-20-1	Ideal S Die Ekstra	<66	17	<52	<80	<36	<30	11	<21	<56	<12	<40	<12	<190	<24
2019-21-32-1	Opti Vital Pluss	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-33-1	Opti Lakta	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-69-1	Vekst 115 Soft Spes	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-70-1	Format Komplett III	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-71-1	Format Kvikk 140	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-78-1	Format Vekst 110	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-81-1	Format Vekst 120	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-21-104-1	Ideal Junior	<66	<16	<52	<80	<36	<30	38	<21	<56	<12	<40	<12	<190	<24
2019-21-123-1	Opti Vital Trygg	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24

Appendix Table 4. Results on fungi (total mould, *Penicillium*, *Aspergillus*, Mucorales, yeast; all cfu/g) and mycotoxins (all µg/kg) in 18 individual samples of **farm-mixed feed for rumimants** in 2019. 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, n.a.=not analysed.

ID-nr.	Total moulds	*Penicillium*	*Aspergillus*	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
2019-23-131-1	1100000	1100000	<50	50000	1400000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-132-1	23000	23000	<50	<50	92000000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-191-1	9100	350	450	8200	1800000	<66	<16	<52	<80	47	<30	10	<21	<56	<12	<40	<12	<190	<24
2019-23-197-1	4500	350	4500	<50	380000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-197-2	n.a.	n.a.	n.a.	n.a.	n.a.	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-229-1	680	500	100	<50	250000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-229-2	550	500	<50	<50	150000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-230-1	260	260	<50	<50	610000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-230-2	320	320	<50	<50	530000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-231-1	11000	10000	<50	1000	5500000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-231-2	10000	8200	<50	500	450000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-232-1	20000	20000	<50	1500	430000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-232-2	20000	15000	<50	5000	650000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-285-1	50	<50	<50	50	47000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-304-1	<50	<50	<50	<50	130000000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-315-1	21000	21000	<50	<50	20000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-316-1	120000	120000	<50	<50	50000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24
2019-23-323-1	<50	<50	<50	<50	700000	<66	<16	<52	<80	<36	<30	<10	<21	<56	<12	<40	<12	<190	<24

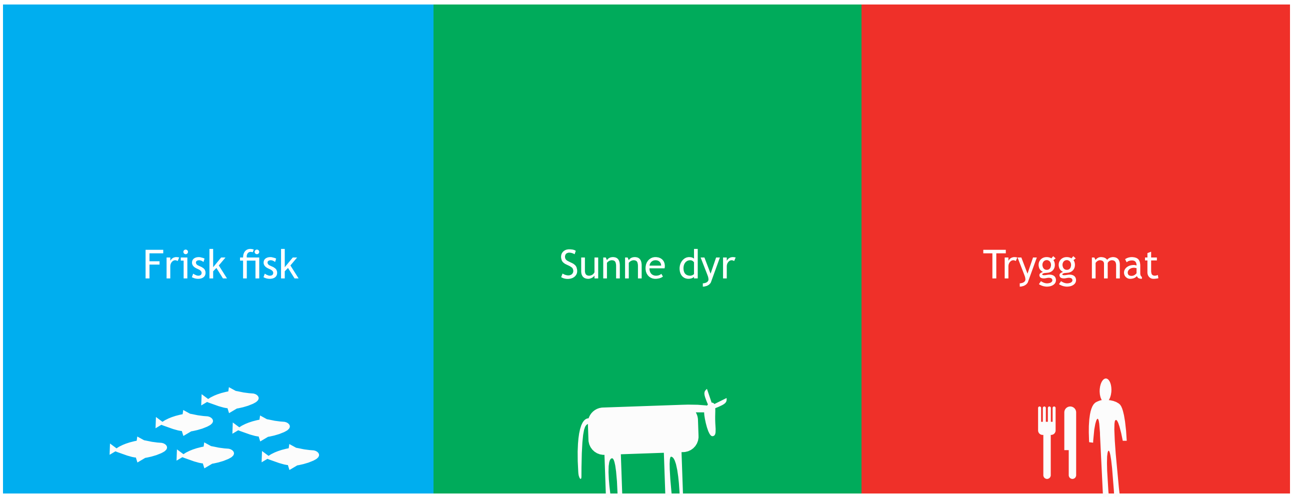
Appendix Table 5. Results on *Escherichia coli*, *Clostridium perfringens* and *Salmonella* in 74 samples of **raw feed for dogs** 2019. Quantitative results in cfu/g. n.d.=not detected.

ID-nr.	Type of feed	Origin	*Escherichia coli*	*Clostridium pefringens*	*Salmonella*
2019-22-8-1	On Track Mix	Norway	160	<10	n.d.
2019-22-9-1	Vom Active	Norway	10	<10	n.d.
2019-22-10-1	Mush Vaisto Adult	Finland	1 600	10	n.d.
2019-22-11-1	Mush Vaisto Puppy	Finland	20	<10	n.d.
2019-22-12-1	On Track Mix	Norway	600	40	n.d.
2019-22-20-1	Mush Vaisto Adult	Finland	160	20	n.d.
2019-22-21-1	Vom Active	Norway	20	20	n.d.
2019-22-30-1	Provit Kylling	Norway	90	20	n.d.
2019-22-31-1	Mush Vaisto Adult	Finland	3 200	<100	n.d.
2019-22-42-1	Mush Vaisto Grønn	Finland	40	<10	n.d.
2019-22-43-1	Vom Digestive	Norway	10	10	n.d.
2019-22-51-1	Natures Menu Country Hunter	England	<10	<10	n.d.
2019-22-195-1	Vom Taste And	Norway	100	10	n.d.
2019-22-196-1	Vom Digestive	Norway	170	10	n.d.
2019-22-197-1	Vom Active m/laks	Norway	320	<10	n.d.
2019-22-198-1	Vom Active kylling	Norway	80	20	n.d.
2019-22-199-1	Vom Taste kylling	Norway	180	<10	n.d.
2019-22-416-1	Rått hundefôr fra slakteri	Norway	150	1 400	n.d.
2019-22-420-1	VIP Jegerpølse	Norway	<10	<10	n.d.
2019-22-462-1	Natures Menu Country Hunter	England	<10	<10	n.d.
2019-22-463-1	Natures Menu Country Hunter	England	40	<10	*S.* Typhimurium
2019-22-464-1	Mush Vaisto Complete Meal	Finland	60	<10	n.d.
2019-22-465-1	Mush Vaisto Puppy	Finland	430	<10	n.d.
2019-22-466-1	Vom Taste 100 % storfevom	Norway	280	<10	n.d.
2019-22-467-1	On Track Hakk	Norway	290	40	n.d.
2019-22-468-1	On Track Pølse	Norway	70	100	n.d.
2019-22-469-1	On Track Mix Pølse	Norway	860	80	n.d.
2019-22-470-1	Våtfôr til hund	Norway	<10	<10	n.d.
2019-22-767-1	Vom Taste 100 % storfevom	Norway	380	<10	n.d.
2019-22-782-1	Rått hundefòr fra slakteri	Norway	300	80	n.d.
2019-22-792-1	Kalkunhals	Norway	560	<10	n.d.
2019-22-793-1	Kyllinghals	Norway	90	<10	n.d.
2019-22-794-1	Vom Puppy pølse	Norway	150	10	n.d.
2019-22-795-1	Provit kylling	Norway	<10	<10	n.d.
2019-22-796-1	Storfevom, kvernet	Norway	500	<10	n.d.
2019-22-797-1	Storfevom, kvernet	Norway	1 500	<10	n.d.
2019-22-798-1	Storfevom, kvernet	Norway	5 300	<10	n.d.
2019-22-820-1	Mush Vaisto Adult, grønn	Finland	10	<10	n.d.
2019-22-821-1	Slakteavfall kateg.3, nyrer gris	Norway	<10	<10	n.d.
2019-22-865-1	Vom Active Hundepølse	Norway	60	<10	n.d.
2019-22-866-1	Vom Taste Hundepølse	Norway	18	<10	n.d.
2019-22-867-1	Vom Taste Hundepølse	Norway	<10	<10	n.d.
2019-22-1005-1	Mush Vaisto Adult	Finland	50	<10	n.d.
2019-22-1006-1	Mush B.A.R.F Basic	Finland	<10	<10	n.d.
2019-22-1007-1	Provit storfevom	Norway	<10	<10	n.d.
2019-22-1080-1	Vom Puppy	Norway	10	27	n.d.
2019-22-1081-1	Vom Active	Norway	750	<10	n.d.
2019-22-1110-1	Vom Taste	Norway	<10	<10	n.d.
2019-22-1179-1	Provit rå kalkunhals	Norway	10	<10	n.d.
2019-22-1180-1	Provit frossen storfevom	Norway	<10	10	n.d.

2019-22-1215-1	Vom Taste	Norway	<10	<10	n.d.
2019-22-1216-1	Vom Active	Norway	10	18	n.d.
2019-22-1217-1	Vom Taste	Norway	<10	<10	n.d.
2019-22-1280-1	Kylling og svin hamburger	Norway	320	10	n.d.
2019-22-1281-1	Kylling og svin hamburger	Norway	220	70	n.d.
2019-22-1282-1	Kylling hamburger	Norway	180	30	n.d.
2019-22-1431-1	Provit Storfevom	Norway	30	<10	n.d.
2019-22-1432-1	On Track Mix	Norway	<10	20	n.d.
2019-22-1433-1	Mush Barf Basic	Finland	170	<10	n.d.
2019-22-1434-1	Hundepølse	Norway	<10	<10	n.d.
2019-22-1445-1	Kvernet vom	Norway	20	<10	n.d.
2019-22-1446-1	Kvernet vom	Norway	1 500	<10	n.d.
2019-22-1492-1	Mush Vaisto Puppy	Finland	<10	<10	n.d.
2019-22-1493-1	Mush Vaisto Adult	Finland	<10	<10	n.d.
2019-22-1494-1	Natures Menu Country Hunter	England	<10	<10	n.d.
2019-22-1495-1	Natures Menu Country Hunter	England	<10	<10	*S.* Derby
2019-22-1559-1	Vom Puppy	Norway	10	40	n.d.
2019-22-1560-1	Vom Active	Norway	40	<10	n.d.
2019-22-1561-1	Mush Wild Adult	Finland	8 800	<10	n.d.
2019-22-1563-1	Provit 3 kg	Norway	80	<10	n.d.
2019-22-1565-1	Mush Vaisto Adult	Finland	<10	<10	n.d.
2019-22-1566-1	Storfèvom	Norway	<10	<10	n.d.
2019-22-1567-1	Innmat storfe	Norway	<10	<10	n.d.
2019-22-1655-1	Smaak Raw Beef, pølse	Finland	<10	<10	n.d.

Appendix Table 6. Results on aflatoxin B1, B2, G1, G2 (µg/kg) in 10 individual samples of **maize** 2019.

ID-nr.	Type	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2
2019-21-9-1	Maize gluten	3.14	<0.10	<0.20	<0.15
2019-21-12-1	Whole maize	<0.25	<0.10	<0.20	<0.15
2019-21-13-1	Organic maize	<0.25	<0.10	<0.20	<0.15
2019-21-13-2	Organic maize	<0.25	<0.10	<0.20	<0.15
2019-21-63-1	Whole maize	<0.25	<0.10	<0.20	<0.15
2019-21-74-1	Maize gluten	15.28	2.02	<0.20	<0.15
2019-21-110-1	Whole maize	<0.25	<0.10	<0.20	<0.15
2019-21-124-1	Whole maize	<0.25	<0.10	<0.20	<0.15
2019-21-125-1	Whole maize	<0.25	<0.10	<0.20	<0.15
2019-21-126-1	Whole maize	<0.25	<0.10	<0.20	<0.15



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