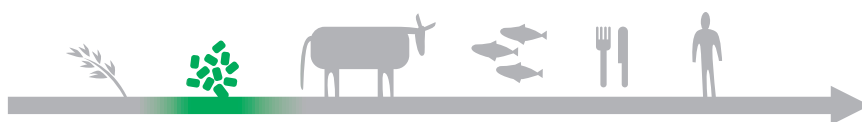


# The surveillance programme for feed materials, complete and complementary feed in Norway 2016 - Mycotoxins, fungi and bacteria



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## Summary

In 2016 various mycotoxins and total mould and *Fusarium* were measured in oats and barley, aflatoxins were measured in maize and compound feed for ruminants, various mycotoxins in compound feed for pig and dog. In addition, pathogenic and hygiene indicating bacteria were determined in raw feed for dog based on residual products from the slaughter industry. A novel multi-mycotoxin method was applied for the simultaneous measurement of a selected repertoire of mycotoxins, which is broader than required by the Norwegian Food Safety Authority. In oats, 58 and 44 % of the samples had higher concentrations than the guidance values of total mould and total *Fusarium*, respectively. The situation was better for barley. The proportion barley samples with total mould and total *Fusarium* higher than guidance values were 17 and 19 %, respectively. These values of mould and *Fusarium* in barley were significantly less than in oats (p-values <0.0001 and =0.005, respectively), and, respectively. The level of storage fungi in oats and barley was similar and few samples were higher than the guidance value.

Oats had higher levels of trichothecene mycotoxins than in barely. Deoxynivalenol (DON), as well as T-2 and HT-2 toxin were the major toxins detected. Six percent of the oat samples, exceeded the guidance value for T-2 + HT-2 in cereal products (500 µg/kg). The trichothecene repertoire expanded in 2016 to include DON and T-2/HT-2 metabolites. DON-3-glucoside was found in 19 % of the oats and 44 % of the barley samples, while lower levels of acetylated DON were found generally. The levels of DON and DON-3-glucoside was significantly correlated. The level of T-2 tetraol was almost similar to that of T-2 or HT-2. Comparing the results of 15 years of surveillance the cereal contamination level of DON and T-2 and HT-2 in 2016 may be characterised as moderate. Concerning regional differences, T-2 and HT-2, were not detected in samples from Region Midt (Trøndelag, Møre and Romsdal), which is consistent with the results from previous surveys.

Zearalenone was only detected in a few cereal samples and far below the recommended limit in feed materials, and its metabolites were hardly detected. Detection of ergot alkaloids was sporadic in the cereal grain samples, more often and at higher maximum concentrations in barley than in oats. The highest level of ergot alkaloids in barley was nearly high enough to elicit clinical effects in livestock animals. In addition, enniatins and moniliformin were more widespread and were at higher levels in barley than in oats. However, the levels are not considered to be high enough for health concern. The *Aspergillus* mycotoxin sterigmatocystin was found in 4 % of the barley samples. Like aflatoxins, this toxin has been shown to be genotoxic *in vitro*. Traces of aflatoxins were found in maize, but no aflatoxins were detected in complementary compound feed for ruminants. The surveillance of aflatoxins is mainly to monitor and control the risk for aflatoxin metabolites in animal products for human consumption.

In compound feed for pigs, DON was detected in most samples but below the recommended level. DON-metabolites were not detected. T-2 and HT-2 were present in 36 % of the pig feed samples, with one sample being above recommended level. T-2 metabolites were not detected. Zearalenone was below the recommended level in all samples of pig feed. However, one sample had high level of alpha-zearalenol which holds higher estrogenic activity than zearalenone. In addition, this feed sample also contained T-2+HT-2 above the recommended level. High levels of these compounds can influence reproduction of sows and general performance and health of growing pigs. Fumonisin and ochratoxin A were below recommended levels in all samples. Ergot alkaloids were not detected in any of the pig feed samples.

In compound feed for dog, DON, T-2+HT-2, zearalenone and ochratoxin A were generally detected, but all samples were below recommended levels.

The raw feed for dogs were analysed for *Salmonella* sp., *Clostridium perfringens* and *Escherichia coli*. *Salmonella* bacteria (*S. Mbandaka*) was found in one of 68 samples. A total of five samples had numbers of *C. perfringens* above 100 cfu/g (maximum 1000 cfu/g). The number of *E. coli* in the samples was highly variable, with 11 samples exceeded 1000 cfu/g (maximum 70,000 cfu/g). The presence of *C. perfringens* has the potential of causing haemorrhagic enteritis in dogs, and *C. perfringens* and *E. coli* in raw materials is indicators of faecal contamination.

## Introduction

The surveillance programme for mycotoxins and fungi in feed materials and complete feed is a collaboration between the Norwegian Food Safety Authority (NFSA) and the Norwegian Veterinary Institute (NVI) where NFSA decides the extent of the programme based on scientific advices from NVI. NFSA is responsible for collecting the samples and NVI for analysing and reporting of the results.

Genus *Fusarium* is the most important mycotoxin-producing moulds primarily infecting cereals in the field during the growing season. They produce important mycotoxins like trichothecenes deoxynivalenol (DON), T-2 toxin and HT-2 toxin, as well as zearalenone and fumonisins. Two decades of surveillance in Norwegian cereals have found DON to occur in high concentrations, particularly in oats and wheat. DON is considered as a health risk if ingested by animals and humans [1]. Exposure to DON causing gastrointestinal signs such as reductions of feed intake and growth rate are well documented in pigs. T-2 and HT-2 levels causing concern are usually present only in oats and oat products. T-2 and HT-2 have similar but potentially stronger toxic effect than DON, in causing gastrointestinal lesions as well as immune suppression [1]. The oestrogenic mycotoxin zearalenone is produced by the same *Fusarium* species as DON, but its level has been insignificant in Norwegian cereals based on limited data on their occurrence [1]. Fumonisins, with varying toxic effects in different animal species are possible human carcinogens [1]. They are primarily produced by *Fusarium* species which infect maize. Enniatins and beauvericin are also *Fusarium* mycotoxins. They have ionophoric and cytotoxic properties but rather low bioavailability via oral exposure. Their animal and human toxic effects are considered low and unknown [1]. Other *Fusarium* mycotoxins include moniliformin, 5-acetamidobutenolid and 15-hydroxy-culmorin for which their toxic profiles are rather unknown.

Ergot alkaloids are emerging mycotoxins of considerable interest in EU and data on their occurrence are of great interest [2]. They cause moderate acute toxicity, such as produce neurotoxicity, inhibit blood circulation and can interfere with hormone levels. The producer of ergot alkaloids, *Claviceps purpurea*, is mainly found in rye.

Species of genera *Penicillium* and *Aspergillus* are the most important mycotoxin-producing moulds that infect cereals and feed primarily during storage. *Penicillium* species that generally grow and produce mycotoxins at lower temperatures than species of *Aspergillus*, are therefore the main concern under Norwegian storage conditions. Ochratoxin A is an important mycotoxin produced by several species of both *Penicillium* and *Aspergillus* worldwide. The most prominent livestock effect of ochratoxin A is nephrotoxicity in pigs. The toxin may also suppress the immune function and performance [1]. So far, ochratoxin A has not caused problems for Norwegian husbandry. Nevertheless, active surveillance of ochratoxin is important because its potential risk and occurrence in imported feed ingredients is unpredictable [1]. Aflatoxins, produced by some *Aspergilli*, are considered a possible import problem for Norway. To minimize the human health risk via consumption of animal products, the carcinogenic and liver toxic aflatoxins in feed must be kept at low levels. Case in point is that aflatoxins in feed for dairy cattle can lead to the presence of an active aflatoxin metabolite in milk. Sterigmatocystin is another mycotoxin produced by *Aspergilli* that may grow in temperate regions. It has similar genotoxic properties as aflatoxins.

Raw feed for dogs are popular and there is little knowledge of the bacteriological/hygienic quality of such products. In this project it was decided to analyse for *Salmonella* which is an important pathogen and a zoonotic bacteria. *Clostridium perfringens* is an obligate anaerobic bacterium that inhabits the colon of healthy animals in low numbers. It has a rapid growth rate and a producer of potent exotoxins, and has the potential of causing haemorrhagic enteritis in dogs. Its presence in raw materials is an indication of faecal contamination. *E. coli* is commonly enumerated as an indicator of faecal contamination and may give indications of the hygienic quality of the raw material and the production hygiene.



## Aims

The aims of the programme are to provide reliable documentation on the occurrence of important mycotoxins and selected fungi and contagious bacteria in feed cereal materials, and complete and complementary feed in Norway, and to use the data 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 2016 the surveillance programme for feed consisted of the following samples received at NVI and analysed for various agents:

- a) 105 samples of small cereal grain consisting of oats (52) and barley (53),
- b) 10 samples of maize/maize gluten,
- c) 46 samples of complementary compound feed for ruminants,
- d) 25 samples of complete compound feed for pigs,
- e) 19 samples of complete compound feed for dogs and
- f) 68 samples of raw for dogs.

The planned sample number of the various categories were as follows:

- a) 100 samples of small cereal grain consisting of oats and barley,
- b) 15 samples of maize/maize gluten,
- c) 50 samples of complementary compound feed for ruminants,
- d) 25 samples of complete compound feed for pigs,
- e) 25 samples of complete compound feed for dogs and
- f) 80 samples of raw for dogs.

Thus, the analysed number of small cereal grains exceeded the sampling plan, whereas most other sample categories were somewhat below the plan, except for feed for pigs which followed the plan exactly.

The samples were collected by NFSA according to the sampling plan. Small grains collected at mills in grain production areas were sent to NVI from September to December. Maize were collected from batches imported from 3rd countries and were received at NVI during February to December. Samples of compound feed for ruminants and pigs collected from the feed industries, and samples of compound feed and raw feed for dogs from pet stores were received by NVI throughout the year. Sampling was according to EU Regulation 691/2013 to ensure samples were representative. Sampling procedures considered factors such as size/volume of the lot to be sampled, uniformity in distribution of the substances to be analysed, number of incremental samples, sampling tools, size of final samples etc.

All samples of cereal grain (oats, barley) were analysed for total mould, *Fusarium* and storage mould, and various mycotoxins by multi-toxin method at NVI. The mycotoxins include trichothecenes, zearalenone and metabolites, ergot alkaloids, enniatins and others (see below).

The maize samples and the samples of compound feed for ruminants were analysed for aflatoxins at NVI.

The compound feed for pigs were either analysed for trichothecenes and zearalenone at Premier Analytical Services in United Kingdom (9 samples), or for these mycotoxins and several others by the multi-toxin method at NVI (16 samples). In addition, ochratoxin A was analysed in all samples at NVI.

The compound feed for dogs were either analysed for trichothecenes and zearalenone at Premier Analytical Services in United Kingdom (13 samples), or for these mycotoxins and several others by the multi-toxin method at NVI (6 samples). In addition, ochratoxin A was analysed in all samples at NVI.

The raw feed for dogs was analysed for *Salmonella* sp., *Clostridium perfringens* and *Escherichia coli* at NVI.

## Quantitative determination of total mould, *Fusarium* and storage fungi

Quantitative determinations by NMKL method No 98 of mould, *Fusarium* and storage fungi in oats and barley were done at NVI where only Malt-yeast-extract-sucrose-agar (MYSA) was used as growth medium. In addition to the total amount of mould a qualitative determination of the composition of the mycoflora was examined. *Fusarium* and storage fungi were counted separately. The detection limit for mould, *Fusarium* and storage fungi in feed is 50 colony forming units per gram (cfu/g).

## Chemical analysis

*Analytical method at Premier Analytical Services in UK*

The trichothecene mycotoxins DON, 3-acetyl-DON, 15-acetyl DON, nivalenol, T-2 toxin and HT-2 toxin were determined by gas chromatography with mass spectrometry (GC/MS). Zearalenone was analysed using immunoaffinity columns for clean-up followed by determination by HPLC with fluorescence detection. Limit of detections: All trichothecenes: 10 µg/kg, zearalenone: 4 µg/kg.

*Analytical methods at NVI*

The novel multi-mycotoxin liquid chromatography-high-resolution mass spectrometry (LC-HRMS/MS) method was applied for the simultaneous determination of selected mycotoxins in the various cereal samples. The developed LC-HRMS/MS multi-mycotoxin method was validated in order to ensure the quality and reliability of collected data. The performance parameters linearity, selectivity, limit of detection (LODs) and limit of quantifications (LOQs) were validated. In addition, spike recovery experiments were performed. The evaluation of matrix effects was performed by utilizing the signal suppression or enhancement (SSE) approach based on a relative difference of the slope of calibration curves constructed with and without matrix extract. The matrix effects were observed for all selected mycotoxins, varying from 64 to 148 %. Reasonable levels of signal suppression or signal enhancement was achieved for only 30 % of targeted mycotoxins. Therefore, in order to control the observed matrix effects, calibrations were based on matrix-assisted standards. With the intention of further improving the accuracy of the method, we introduced stable-isotope labelled internal standards for seven of the most important mycotoxins including DON, 3-acetyl-DON, nivalenol, HT-2, T-2, zearalenone and ochratoxin A.

In order to improve the extraction methodology with respect to polar and nonpolar compounds, a two-step extraction was carried out (MeCN:H<sub>2</sub>O:HCOOH, 80:19.9:0.1, v/v/v and MeCN:H<sub>2</sub>O:HCOOH, 20:79.9:0.1, v/v/v). 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 an UHPLC Dionex Ultimate 3000 system (Thermo FisherScientific).

*Analytes and limit of detections*

DON: 14 µg/kg, 3-acetyl-DON: 6 µg/kg, 15-acetyl-DON: 40 µg/kg, DON-3-glucoside: 26 µg/kg, T-2 toxin: 7 µg/kg, HT-2 toxin: 14 µg/kg, T-2 triol: 80 µg/kg, T-2 tetraol: 16 µg/kg, nivalenol: 100 µg/kg, zearalenone: 36 µg/kg, alpha-zearalenol: 15 µg/kg, beta-zearalenol: 11 µg/kg, ergonovine: 50 µg/kg, ergosine: 80 µg/kg, ergotamine: 80 µg/kg, ergocornine: 80 µg/kg, alpha-ergocryptin: 80 µg/kg, ergocristin: 80 µg/kg, fumonisin B1: 80 µg/kg, fumonisin B2: 80 µg/kg, enniatin A: 13 µg/kg, enniatin A1: 14 µg/kg, enniatin B: 25 µg/kg, enniatin B1: 27 µg/kg, beauvericin: 14 µg/kg, moniliformin: 20 µg/kg, 5-acetamidobutenolol: 64 µg/kg, 2-amino-14,16-dimetyloctadecan-3-ol (2-AOD-3-ol): 200 µg/kg, 15-hydroxyculmorin: 11 µg/kg, neosolaniol: 15 µg/kg, fusarin X: 18 µg/kg, alternariol: 48 µg/kg, alternariol methylether: 46 µg/kg, sterigmatocystin 12 µg/kg.

Ochratoxin A was analysed using immunoaffinity clean-up followed by determination by HPLC with fluorescence detection. The limit of detection for ochratoxin was 0.01 µg/kg.

Aflatoxins (B1, B2, G1, G2) were analysed using immunoaffinity columns clean up followed by determination by HPLC using fluorescence detection after post-column derivatisation. The detection limit 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

The samples of raw dog food were collected by NFSA and sent to the laboratory frozen. The samples of raw dog food were analysed for *Salmonella* by using the ISO 6579:2002/Amd.1:2007: Annex D (Detection of *Salmonella* spp. in animal faeces and in samples from the primary production stage). Briefly, the samples were enriched in Buffered Peptone water (BPW) prior to selective enrichment on modified semi solid Rappaport Vassiladis agar (MSRV). After incubation, colony material from MSRV plates with typical appearance, were plated onto XLD and BGA plates. Typical and suspect colonies were selected for biochemical testing, identification by MALDI-TOF and eventually serotyping.

Analysis for *Clostridium perfringens* were carried out using NMKL no. 95, 5<sup>th</sup> ed. 2009 (*Clostridium perfringens*. Determination in foods, feed and environmental samples). Aliquots of the appropriate dilutions of the samples were pour-plated on TSC-medium for enumeration of *Cl. perfringens* (detection limit of 10 cfu/g). Typical colonies were selected for biochemical tests and identification by MALDI-TOF. The first two samples were analysed by plating directly on sheep blood agar with a detection limit of 100 cfu/g. However, this approach was stopped and the NMKL method was applied for the remaining samples.

*E. coli* was enumerated by the use of 3M™ Petrifilm™ Select *E. coli* following the manufacturer's instructions. *E. coli* was enumerated from refrozen samples as it was decided during the project that the samples would be analysed quantitatively for *E. coli*.

## Statistical analysis

Descriptive statistics using mean and standard deviation were employed to estimate the true population mean and variance for the various contaminants with levels measured in a quantitative manner. One way Anova was used to determine significance in statistical difference between feed types for the same contaminant. To investigate possible linear correlation between to contaminants in the same feed type, correlation and regression coefficients were estimated and their p value determined.

## Results and discussion

### Cereals

#### *Fungi in oats and barley*

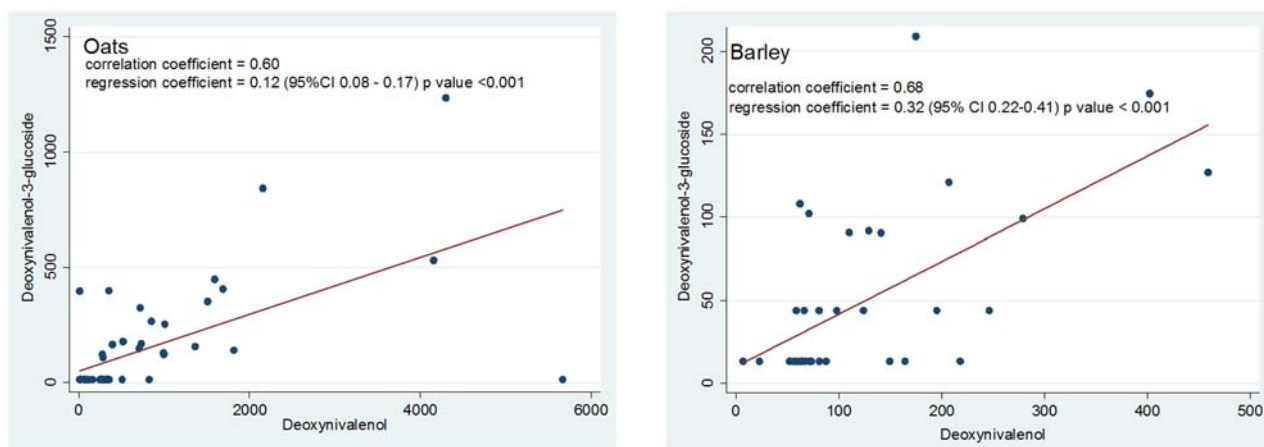
The concentrations of total mould and total *Fusarium* in oats were above guidance values (350,000 and 25,000 cfu/g, respectively) in 58 and 44 % of the samples (Table 1). In spite for the many samples exceeding guidance values, the fungal quality of oats was at an average level compared with mean mould and *Fusarium* concentrations observed during the last decade [3]. Concerning storage fungi, the situation was better with 6 % of the oat samples above guidance value (25,000 cfu/g). Barley had significantly less total mould and total *Fusarium* than in oats (p-values <0.0001 and =0.005, respectively). The percentages of barley samples exceeding guidance levels for total mould and *Fusarium* were 17 and 19 %. The level of storage fungi in barley was similar as in oats.

#### *Trichothecenes in oats and barley*

As usual, levels of trichothecenes were higher in oats than in barely (Table 1). In previous NVI reports on mycotoxin surveillance in feed, the trichothecenes analysed consisted of DON, T-2, HT-2 and nivalenol. For 2016, analysis also included 3- and 15-acetyl-DON, DON-3-glucoside, T-2 triol and T-2 tetraol. In both oats and barley, DON was the highest in concentration. In addition, DON-metabolites were detected in many samples of oats and barley. Compared with levels of DON, 19 and 44 % DON-3-glucoside were found in oats and barley. DON and DON-3-glucoside were positively significantly correlated (p<0.001) in both barley and oats (Figure 1). Lower levels were found of acetylated DON metabolites. The relative levels of DON-metabolites were similar to findings of Uhlig et al [4] in Norwegian oats and barley in 2011 - a year with considerable cereal DON levels.

In oats and barley DON levels in 2016 were respectively four times and two times higher than in 2015 [5]. Based on the last fifteen years of surveillance of levels of contamination in cereals, levels of DON in 2016

was moderate (Figure 2). No samples exceeded the recommended limit for feed material by EU and Norway (8000 µg/kg) [6, 7]. The DON concentrations in cereal were evenly spread in all regions. Regional differences of DON concentrations could not be determined given the small sample sizes.



**Figure 1.** Correlation between DON and DON-3-glucoside in oats (N=52) (left) and barley (N=53) (right). A regression line is fitted to the points and that allows predictions of levels of DON-3-glucoside 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 [7, 8] were found in nearly half of the samples of oats and some samples of barley. Six percent of the oat samples, exceeding the guidance value for T-2 + HT-2 in cereal products (500 µg/kg). The contamination level of T-2 and HT-2 in 2016 was moderate and lower than in 2015 (Table 1, Figure 2). T-2 and HT-2 were unquantifiable in cereal samples from Region Midt (Trøndelag, Møre and Romsdal), consistent with the results from previous surveys [5, 9].

**Table 1.** Concentrations (cfu/g) of fungi (total mould, total Fusarium and storage fungi) and concentrations (µg/kg) of trichothecenes (deoxynivalenol (DON), 3-acetyl-DON, 15-acetyl- DON, DON-3-glucoside, sum of T-2 and HT-2 toxin and T-2 tetraol) and zearalenone (ZEA) in oats and barley sampled in Norway in 2016.

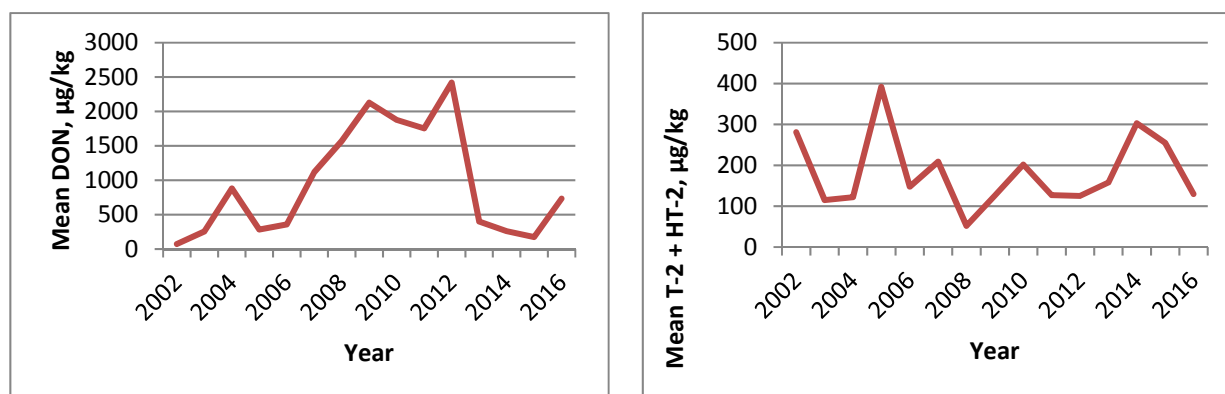
	Total mould	Total <i>Fusarium</i>	Storage fungi	DON	3-ADON	15-ADON	DON-3-G	T2+HT-2	T-2 tetra	ZEA
<b>Oats (n = 52)</b>										
Mean	620,000	40,800	5,600	734	67	<40	140	130	38	<36
Median	450,000	25,000	<50	325	31	<40	<26	<20	<17	<36
Min-max	4,100 - 2,600,000	<50 - 180,000	<50 - 100,000	<14 - 5,670	<6 - 541	<40 - 361	<26 - 1,230	<20 - 1,550	<17 - 498	<36 - 268
St. dev.	575,000	43,200	17,000	1,130	99	54	232	263	100	45
% samples above detection limit	100	96	29	87	65	4	40	46	12	10
% samples above guidance values	58	44	6	0				6		0
<b>Barley (n = 53)</b>										
Mean	230,000	20,700	7,550	87	<6	<40	38	<20	<17	<36
Median	140,000	10,000	<50	62	<6	<40	<26	<20	<17	<36
Min-max	9,100 - 2,000,000	1,400 - 160,000	<50 - 210,000	<14 - 459	<6 - 25	<40	<26 - 209	<20 - 122	<17 - 176	<36 - 286
St. dev.	298,000	26,100	29,200	97	4	0	45	15	26	37
% samples above detection limit	100	100	45	74	15	0	32	4	11	6
% samples above guidance values	17	19	2	0				0		0



The metabolite T-2 triol was found in several samples and at a similar level as T-2 or HT-2. This is the same findings as presented by Uhlig et al [4] for Norwegian cereal in 2011. T-2 triol was not detectable in the cereal samples. Nivalenol was also hardly detected.

The moderate contamination level of both DON and T-2 + HT-2 in 2016 could be due to the fair weather experienced during the growth season in the cereal producing regions [10]. *Fusarium graminearum* is the main producer of DON and *F. langsethiae* the main producer of HT-2 and T-2 in Norwegian cereals [1]. Several scientists have opined *F. graminearum* and *F. langsethiae* are mutually exclusive, and that *F. langsethiae* is more predominant during dryer summers [11, 12]. The mechanisms behind this are not fully understood, however, some data indicate that conditions favouring *F. graminearum* will repress *F. langsethiae*, whereas unfavourable conditions and absence of *F. graminearum*, will allow for *F. langsethiae* to proliferate [13].

Figure 2 illustrate a kind of inverse relationship between DON and HT-2 + T-2 in oats during 2002-16. Thus, in “good” years where DON contamination is low, there may be a reason to expect increased problems with HT-2/T-2 contamination. It also suggests that a one-sided focus on eradication of *F. graminearum*, may result in a proliferation and increased problems with *F. langsethiae*. This situation is supported by the observation that currently there is no fungicide that has proven effective against *F. langsethiae* [14].



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

#### *Other mycotoxins in oats and barley*

Zearalenone was only detected in a few cereal samples at levels that were far below the recommended limit in feed materials (2000 µg/kg) in EU and Norway [6, 7]. The metabolites alpha-zearalenol and beta-zearalenol were only detected in a single sample - the oat sample which also showed the highest concentration of zearalenone. The sum of all these three compounds was 533 µg/kg.

Ergot alkaloids were sporadically detected in the cereal grain samples - more often and at higher maximum concentrations in barley than in oats. Unfortunately, data on toxic effects for risk assessment are lacking. Dose-response studies using ergot alkaloids have not shown major quantitative difference in toxicity between the compounds in rats, with no-observed-adverse-effect-levels (NOAELs) of sum ergot alkaloids in the region 0.22-0.60 mg/kg b.w. per day [2]. This corresponds to 3-7 mg/kg dried feed. Few dose-response studies have been conducted in livestock animals. Reduced feed intake is shown in piglets fed total alkaloids in the range 3.6-11 mg/kg dried feed. In chicken, intestinal inflammation was induced when fed 2.8 mg/kg dried feed and above [2]. The maximum higher levels of ergot alkaloids detected in our barley samples were nearly high enough to elicit clinical effects in livestock animals.

Enniatins and moniliformin were more widespread and were at higher concentrations in barley than in oats. Enniatin B and enniatin B1 were the enniatins with dominating occurrence. The enniatin and moniliformin levels in oats and barley were within the range of previous and limited Norwegian data [1, 4]. The hazard characterisation of enniatins and moniliformin are inadequate but levels detected are not

considered to be of health concern. 5-acetamidobutenolid and 15-hydroxy-culmorin were detected in a few samples. Their toxic potency is unknown.

The *Aspergillus* mycotoxin sterigmatocystin was found in two samples of barley (20 and 225 µg/kg). This toxin is genotoxic *in vitro* and is comparable with the aflatoxins, but seems to be less hepatocarcinogenic [15]. A maximum limit for this toxin is lacking, but for comparison, the maximum limit for aflatoxin B1 in cereals is 2.0 µg/kg. The other *Fusarium* metabolites neosolaniol, fusarin X, beauvericin, 2-amino-14,16-dimetyloctadecan-3-ol (2-AOD-3-ol), alternariol and alternariol methylether were not detected in the cereal samples.

**Table 2.** Regional survey of fungi (total mould, total *Fusarium* and storage fungi all cfu/g) and trichothecenes (deoxynivalenol (DON), 3-acetyl-DON, 15-acetyl- DON, DON-3-glucoside, sum of T-2 and HT-2 toxin and T-2 tetraol) and zearalenone (ZEA, all concentrations µg/kg) in oats and barley sampled in Norway in 2016.

		Total mould	Fus	Storage fungi	DON	3-ADON	15-ADON	DON-3-G	T-2+ HT-2	T-2 tetra	ZEA
<b>Region Øst (Buskerud, Vestfold, Telemark, Hedmark, Oppland)</b>											
Oats n=13	Mean	542,000	27,500	6,240	290	30	<40	72	37	<17	<36
	St. dev.	426,000	40,200	8,140	417	43	0	100	53	0	0
Barley n=14	Mean	135,000	15,200	21,600	52	<6	<40	27	<20	<17	<36
	St. dev.	109,000	14,300	55,000	43	6	0	52	0	13	0
<b>Region Stor-Oslo (Akershus, Oslo, Østfold)</b>											
Oats n=24	Mean	884,000	43,800	4,250	635	66	<40	149	199	51	<36
	St. dev.	669,000	37,800	14,100	571	76	0	200	345	125	31
Barley n=24	Mean	234,000	18,300	2,500	119	<6	<40	53	<20	20	<36
	St. dev.	175,000	19,900	5,560	94	3	0	46	23	37	9
<b>Region Midt (Nord-Trøndelag, Sør-Trøndelag, Møre og Romsdal)</b>											
Oats n=10	Mean	285,000	53,900	10,600	1,390	121	73	142	<20	<17	47
	St. dev.	275,000	60,200	31,500	1,980	169	117	212	8	0	79
Barley n=10	Mean	112,000	13,600	1,920	70	<6	<40	<26	<20	<17	<36
	St. dev.	159,000	14,700	3,310	140	0	0	36	0	0	0
<b>Region Sør og Vest (Aust-Agder, Vest-Agder, Rogaland, Hordaland, Sogn og Fjordane)</b>											
Oats n=5	Mean	231,000	34,200	620	1,050	63	<40	277	274	108	47
	St. dev.	140,000	39,300	1,330	1,820	94	0	537	270	161	65
Barley n=5	Mean	710,000	62,000	3,830	64	<6	<40	<26	<20	<17	80
	St. dev.	728,000	55,100	7,930	92	0	0	14	0	9	117

**Table 3.** Concentrations of ergotalkaloids (ergonovine, ergosine, ergotamine, ergocornine, alpha-ergocryptine, ergocristine, sum ergot alkaloids (ΣEA), sum of enniatins (ΣEN), moniliformin (MON), 5-acetamidobutenolid (BUT), 15-hydroxy-culmorin (CUL) and sterigmatocystin (STE) in oats and barley sampled in Norway in 2016.

	Enov	Esin	Etam	Ecor	a-Ecry	Ecri	ΣEA	ΣEN	MON	BUT	CUL	STE
<b>Oats (n = 52)</b>												
Mean	<50	<80	<80	<80	<80	<80	<450	126	26	<64	20	<12
Median	<50	<80	<80	<80	<80	<80	<450	<79	<20	<64	<11	<12
Min-max	<50	<80-785	<80	<80	<80	<80-369	<450-970	<79-826	<20-333	<64-1,010	<11-201	<12
St. deviation	0	103	0	0	0	46	112	152	51	138	40	0
% samples above detection limit	0	2	0	0	0	2	4	73	13	6	17	0
<b>Barley (n = 53)</b>												
Mean	<50	<80	<80	<80	<80	<80	<450	1 216	76	<64	13	<12
Median	<50	<80	<80	<80	<80	<80	<450	939	<20	<64	<11	<12
Min-max	<50	<80-120	<80	<80-1,030	<80-966	<80-326	<450-2,221	<79-4,871	<20-390	<64-280	<11-116	<12-225
St. deviation	0	19	0	136	127	42	278	901	109	51	20	30
% samples above detection limit	0	6	0	2	2	6	4	98	47	23	21	4

**Table 4.** Regional survey of the concentrations of ergotalkaloids (ergonovine, ergosine, ergotamine, ergocornine, alpha-ergocryptine, ergocristine, sum ergot alkaloids ( $\Sigma$ EA), sum of enniatins ( $\Sigma$ EN), moniliformin (MON), 5-acetamidobutenolid (BUT), 15-hydroxy-culmorin (CUL) and sterigmatocystin (STE) in oats and barley sampled in Norway in 2016.

		Enov	Esin	Etam	Ecor	a-Ecry	Ecri	$\Sigma$ EA	$\Sigma$ EN	MON	BUT	CUL	STE
<b>Region Øst (Buskerud, Vestfold, Telemark, Hedmark, Oppland)</b>													
<b>Oats</b> n=13	Mean	<50	<80	<80	<80	<80	<80	<450	124	<20	<64	<11	<12
	St. dev.	0	0	0	0	0	0	0	95	26	0	0	0
<b>Barley</b> n=14	Mean	<50	<80	<80	111	106	<80	<450	1,150	38	<64	<11	<12
	St. dev.	0	29	0	265	247	76	535	541	70	20	3	0
<b>Region Stor-Oslo (Akershus, Oslo, Østfold)</b>													
<b>Oats</b> n=24	Mean	<50	<80	<80	<80	<80	<80	<450	79	<20	<64	18	<12
	St. dev.	0	0	0	0	0	0	0	66	27	0	34	0
<b>Barley</b> n=24	Mean	<50	<80	<80	<80	<80	<80	<450	1,140	80	<64	17	<12
	St. dev.	0	0	0	0	0	0	0	594	99	57	20	0
<b>Region Midt (Nord-Trøndelag, Sør-Trøndelag, Møre og Romsdal)</b>													
<b>Oats</b> n=10	Mean	<50	<80	<80	<80	<80	<80	<450	207	50	<64	41	<12
	St. dev.	0	0	0	0	0	0	0	287	103	71	64	0
<b>Barley</b> n=10	Mean	<50	<80	<80	<80	<80	<80	<450	816	109	68	<11	<12
	St. dev.	0	25	0	0	0	25	51	971	161	68	0	0
<b>Region Sør og Vest (Aust-Agder, Vest-Agder, Rogaland, Hordaland, Sogn og Fjordane)</b>													
<b>Oats</b> n=5	Mean	<50	189	<80	<80	<80	106	<450	187	33	227	30	<12
	St. dev.	0	333	0	0	0	147	329	147	41	436	48	0
<b>Barley</b> n=5	Mean	<50	<80	<80	<80	<80	<80	<450	2,590	99	<64	28	52
	St. dev.	0	0	0	0	0	36	36	1,630	127	34	49	97

#### *Aflatoxins in maize*

The ten maize samples were analysed for aflatoxins. Eight samples did not contain detectable levels of aflatoxin B1, B2, G1 or G2. In two samples were found unquantifiable trace concentrations of aflatoxin B1. Thus, no samples were above the maximum limit.

## Complete and complementary feed

#### *Feed for ruminants*

Complementary compound feed for ruminants were analysed for aflatoxins. None of the 46 samples contained detectable levels. This is a satisfactory result and also consistent with most previous findings for these carcinogenic toxins [1, 3, 5]. The main purpose of the surveillance of aflatoxins in feed for ruminants is to monitor and control the risk of aflatoxin metabolites in milk and dairy products for human consumption.

#### *Feed for pig*

The levels of various mycotoxins in complete compound feed for pigs are shown in Table 5. DON was detected in most samples, but all samples were below the recommended level for feed for pig in Norway (500 µg/kg) [7]. The 3- and 15-acetylated DON-metabolites or DON-3-glucoside were not detected (not shown). The sum of T-2 and HT-2 was present in 36 % of the pig feed samples, and above the recommended level (250 µg/kg) in one sample [7, 8]. The T-2 metabolites T-2 triol and T-2 tetraol were not detected (not shown).

Zearalenone was below the recommended level for pig feed in Norway (250 µg/kg) in all samples [7]. However, it is important to notice that zearalenone metabolites may occur. In this material, one sample showed high concentration of alpha-zearalenol, which holds higher estrogenic activity than zearalenone. No official regulations apply to the metabolites. The feed sample with elevated concentration of the

zearalenone metabolite also contained T-2+HT-2 above recommended level, and could potentially influence reproduction of sows and general performance and health of growing pigs.

Fumonisin and ochratoxin A were below recommended levels for pig feed in Norway in all samples (500 and 10 µg/kg, respectively) [7]. However, it worth to notice that ochratoxin A was detected in all samples.

Enniatins, moniliformin, 15-hydroxy-culmorin and ergot alkaloids are not regulated. Enniatins were detected in most samples, but toxicological data for risk assessment in animal feed is lacking. However, low bioavailability indicates low toxicity, and no risk at the present level. Moniliformin and culmorin were found at low levels not considered to be of any health concern.

Ergot alkaloids were not detected. They were presented in the table as occurrence data are needed [2].

**Table 5.** Concentrations of deoxynivalenol (DON), sum of T-2 and HT-2 toxin (T-2+HT-2), zearalenone (ZEA), alpha- and beta-zearalenol (aZEA, bZEA), sum of ergot alkaloids (ΣEA), sum of fumonisin B1 and B2 (FB1+FB2), sum of enniatins (ΣEN), moniliformin (MON), 15-hydroxy-culmorin (CUL) and ochratoxin A (OTA) in complete compound feed for pigs in 2016.

	DON	T-2+HT-2	ZEA	aZEA	bZEA	ΣEA	FB1+FB2	ΣEN	MON	CUL	OTA
Number	25	25	25	16	15	15	12	16	16	16	25
Mean	71	41	<36	49	<11	<450	<160	256	29	<11	0.59
Median	67	<20	<36	<15	<11	<450	<160	215	<20	<11	0.31
Min-max	<14-168	<20-456	<36-219	<15-529	<11	<450	<160-240	<79-892	<20-185	<11-40	0.01-2.00
St. deviation	35	89	46	133	0	26	50	249	46	11	0.62
% above detection limit	80	36	36	13	0	0	17	?	25	19	100
% samples above guidance values	0	4	0				0				0

#### Feed for dog

DON, T-2+HT-2 and zearalenone were detected in several samples, and ochratoxin A was detected in all samples of complete compound feed for dogs (Table 6). All samples were below recommended levels for dog feed in Norway (DON: 2000 µg/kg, T-2+HT-2: 250 µg/kg, zearalenone: 500 µg/kg, and ochratoxin A: 1000 µg/kg) [7].

Other mycotoxins or metabolites were not detected in six samples analysed, except traces of enniatin B and moniliformin in single samples.

Raw feed for dogs based on residual products from the slaughter industry were analysed for *Salmonella* sp., *Clostridium perfringens* and *Escherichia coli*. *Salmonella* bacteria (*S. Mbandaka*) was isolated from one of 68 samples. The majority of the samples contained 100 or less *C. perfringens*/g. A total of five samples had numbers above 100 cfu/g, of which one had 1000 cfu/g. Elevated levels of *C. perfringens* has the potential of cause haemorrhagic enteritis in dogs. For *E. coli*, however, several of the samples had numbers of *E. coli* above the detection limit in variable numbers. Eleven samples (16 %) had *E. coli* >1000 cfu/g, of which one sample had 70,000 *E. coli*/g. Due to the material tested, it is expected to find *E. coli* in the samples. However, the presence of *E. coli* is used as an indicator of faecal contamination, and high numbers of *E. coli* could be interpreted as poor production hygiene and faecally contaminated raw materials. A level of >1000 cfu/g must be considered as significantly reduced hygienic quality for both *E. coli* and *Cl. perfringens* individually.

**Table 6.** Concentrations of deoxynivalenol (DON), sum of T-2 and HT-2 toxin (T-2+HT-2), zearalenone (ZEA) and ochratoxin A (OTA) in complete compound feed for dogs in 2016.

	DON	T2+HT-2	ZEA	OTA
Number	19	19	19	19
Mean	84	<20	<36	0.26
Median	73	<20	<36	0.20
Min-max	<14-240	<20-55	<36-247	0.01-0.74
St. deviation	79	14	54	0.23
% above detection limit	84	32	63	100
% samples above guidance values	0	0	0	0

**Table 7.** Concentrations of *Clostridium perfringens* and *Escherichia coli* in raw food for dogs in 2016.

	<i>C. perfringens</i>	<i>E. coli</i>
Number	68	68
Mean	54	1,710
Median	<10	65
Min-max	<10 - 1,000	<10 - 70,000
St. deviation	151	8,540
% samples with detectable concentrations	16	87

## Conclusions

### Feed ingredients

- Fungi in oats and barley: In oats, 58 and 44 % of the samples had higher concentrations than the guidance values of total mould and total *Fusarium*, respectively. The corresponding figures in barley were 17 and 19 %. The level of storage fungi in oats and barley was similar and few samples were higher than the guidance value.
- Trichothecenes in oats and barley: Oats had higher levels of trichothecene mycotoxins than in barley. DON and T-2 and HT-2 were the major toxins detected. Six percent of the oat samples, exceeded the guidance value for T-2 + HT-2 in cereal products (500 µg/kg). DON-3-glucoside was found in 19 % of the oats and 44 % of the barley samples, while lower levels of acetylated DON were found generally. The levels of DON and DON-3-glucoside was significantly correlated. Levels of T-2 tetraol was almost similar to that of T-2 and HT-2. Comparing the results of 15 years of surveillance the cereal contamination level of DON and T-2 and HT-2 in 2016 may be characterised as moderate. T-2 and HT-2, were not detected in samples from Region Midt (Trøndelag, Møre and Romsdal), which is consistent with the results from previous surveys.
- Zearalenone in oats and barley: Zearalenone was only detected in a few cereal samples and far below the recommended limit in feed materials.
- Ergot alkaloids in oats and barley: Ergot alkaloids were sporadic in the cereal grain samples, more often and at higher maximum concentrations in barley than in oats. The highest level of ergot alkaloids in barley was nearly high enough to elicit clinical effects in livestock animals.
- Sterigmatocystin in barley: The *Aspergillus* mycotoxin sterigmatocystin was found in 4 % of the barley samples. Like aflatoxins, this toxin has been shown to be genotoxic *in vitro*.
- Aflatoxins in maize: Only traces were found.



## Feed

- Complementary compound feed for ruminants: No aflatoxins were detected.
- Compound feed for pig: DON was detected in most samples but below the recommended level. T-2 and HT-2 were present in 36 % of the samples, with one sample being above recommended level. Zearalenone was below the recommended level in all samples, but one sample had high level of alpha-zearalenol which holds higher estrogenic activity than zearalenone. Fumonisin and ochratoxin A were below recommended levels in all samples, and ergot alkaloids were not detected in any of the pig feed samples.
- Compound feed for dog: DON, T-2+HT-2, zearalenone and ochratoxin A were generally detected, but all samples were below recommended levels.
- Raw feed for dog: *Salmonella* Mbandaka was found in one sample. *C. perfringens*, which has the potential of causing haemorrhagic enteritis in dogs was detected at variable concentrations in several samples, and *E. coli* was found at highly variable concentrations, with 11 samples (16 %) exceeded 1000 cfu/g, indicating significantly reduced hygienic quality. Both *C. perfringens* and *E. coli* in raw materials is an indication of faecal contamination.

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## Appendix

**Appendix Table 1.** Concentrations of fungi (total mould, *Fusarium*, storage fungi) and mycotoxins in oats (52 samples) and barley (53 samples) in individual samples from different districts and regions in 2016. Concentration of fungi in cfu/g. Concentrations of mycotoxins in µg/kg. DON=deoxynivalenol, 3aDON=3-acetyl-DON, 15aDON=15-acetyl-DON, DON3G=DON-3-glucoside, T2=T-2 toxin, HT2=HT-2 toxin, T2tet=T-2 tetraol, NIV=nivalenol, ZEA=zearalenone, aZOL=alpha-zearalenol, bZOL=beta-zearalenol, E.nov=ergonovine, E.sin=ergosine, E.tam=ergotamine, E.cor=ergocornine, aE.cry=alpha-ergocryptine, E.cri=ergocristine, EA=enniatin A, EA1=enniatin A1, EB=enniatin B, EB1=enniatin B1, BEA=beauvericin, MON=moniliformin, BUT=5-acetamidebutenolid, CUL=15-hydroxy-culmorin, FUSX=fusarin X, NEO=neosolaniol, STE=sterigmatocystin.

Jnr	Mould	Fus	Sto	DON	3aDO N	15a DON	DON3 G	T2	HT2	T2 tet	NIV	ZEA	aZOL	bZOL	E.nov	E.sin	Etam	E.cor	aE.cry	E.cri	EA	EA1	EnB	EnB1	BEA	MON	BUT	CUL	FUSX	NEO	STE
<b>OATS Region Midt</b>																															
210-4	64000	64000	<50	5670	168	<40	<26	<7	<14	<17	<100	60	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<64	98	<18	<15	<12
210-5	90 000	25000	<50	1507	119	<40	352	<7	23	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<64	18	<18	<15	<12
210-6	450000	180000	100000	159	<6	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<64	<11	<18	<15	<12
229-1	450000	25000	5000	23	<6	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	234	46	131	92	<64	<11	<18	<15	<12
230-4	4 100	100	<50	127	29	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<64	<11	<18	<15	<12
230-5	30 000	<50	600	265	10	<40	<26	<7	23	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<64	<11	<18	<15	<12
230-6	40 000	15000	<50	341	88	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<64	<11	<18	<15	<12
243-4	590000	50000	<50	4156	541	361	531	<7	<14	<17	<100	268	97	168	<50	<80	<80	<80	<80	<80	<13	<14	<25	597	<14	333	253	201	<18	<15	<12
243-5	360000	140000	<50	1591	245	209	448	<7	23	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	107	56	<18	<15	<12
243-6	770000	40000	<50	61	<6	<40	<26	<7	23	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	402	404	<27	<14	<20	<64	<11	<18	<15	<12
<b>BARLEY Region Midt</b>																															
210-1	60 000	5000	<50	<14	<6	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<64	<11	<18	<15	<12
210-2	68 000	10000	3000	74	<6	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	120	<80	<80	<80	120	<13	<14	141	<27	<14	<20	<64	<11	<18	<15	<12
210-3	86 000	5000	10000	23	<6	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<64	<11	<18	<15	<12
228-1	130000	18000	<50	<14	<6	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	423	46	<14	<20	<64	<11	<18	<15	<12
230-1	9 100	1400	1000	23	<6	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	41	<27	<14	<20	<64	<11	<18	<15	<12
230-2	14 000	1800	<50	<14	<6	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	62	295	115	<14	<20	<64	<11	<18	<15	<12
230-3	68 000	20000	5000	<14	<6	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	72	251	122	<14	<20	<64	<11	<18	<15	<12
243-1	110000	20000	<50	88	<6	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	106	1508	313	<14	290	107	<11	<18	<15	<12
243-2	550000	50000	<50	<14	<6	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	76	1351	215	<14	338	107	<11	<18	<15	<12
243-3	25 000	5000	<50	459	<6	<40	127	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	79	2372	369	<14	390	240	<11	<18	<15	<12
<b>OATS Region Stor-Oslo</b>																															
236-1	220000	20000	1000	262	<6	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	<25	46	<14	<20	<64	<11	<18	<15	<12
237-1	910000	15000	<50	851	<6	<40	267	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	<25	46	<14	<20	<64	<11	<18	<15	<12
239-1	2 100 000	100000	<50	<14	45	<40	397	<7	63	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	<25	46	<14	<20	<64	<11	<18	<15	<12
240-2	1 000 000	15000	<50	321	10	<40	<26	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	<25	46	<14	67	<64	<11	<18	<15	<12
253-1	500000	91000	<50	332	31	<40	<26	99	152	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<64	<11	<18	<15	<12
254-1	450000	36000	<50	507	34	<40	<26	42	114	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	<25	<27	<14	<20	<64	<11	<18	<15	<12
273-1	1 000 000	20000	50000	707	58	<40	148	<7	<14	<17	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<13	<14	41	309	<14	<20	<64	<11	<18	<15	<12
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NORWEGIAN VETERINARY INSTITUTE

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<b>BARLEY Region Stor-Oslo</b>																															
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<b>OATS Region Sør og Vest</b>																															

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<b>BARLEY Region Øst</b>																															
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**Appendix Table 2.** Concentrations of mycotoxins in individual samples of complete feed for pigs (25 samples) in 2016. All concentrations in µg/kg. DON=deoxynivalenol, 3aDON=3-acetyl-DON, 15aDON=15-acetyl-DON, T2=T-2 toxin, HT2=HT-2 toxin, NIV=nivalenol, ZEA=zearalenone, aZOL=alpha-zearalenol, bZOL=beta-zearalenol, E.nov=ergonovine, E.sin=ergosine, E.tam=ergotamine, E.cor=ergocornine, aE.cry=alpha-ergocryptine, E.cri=ergocristine, FB1=fumonisin B1, FB2= fumonisin B2, EA=enniatiin A, EA1=enniatiin A1, EB=enniatiin B, EB1=enniatiin B1, MON=moniliformin, CUL=15-hydroxy-culmorin, OTA=ochratoxin A.

Jnr	Type of feed	DON	3a DON	15a DON	T2	HT2	NIV	ZEA	aZOL	bZOL	E. nov	E. sin	E. tam	E. cor	aE. cry	E. cri	Fum B1	Fum B2	EnA	En A1	EnB	En B1	MON	CUL	OTA	
6-1	Ideal 50	66	<10	<10	<10	<10	<10	4																	1.60	
7-1	Ideal purkefor	63	<10	<10	<10	54	<10	9																		0.11
7-2	Ideal 50	45	<10	<10	12	29	<10	6																		0.03
25-1	Ideal 50	68	<10	10	18	30	12	100																		0.27
26-1	Smågrisfor	14	<10	<10	<10	11	<10	8																		0.03
26-2	Purkefor	47	<10	<10	23	63	13	<3																		0.07
27-1	Format purke	75	<10	<10	37	18	<10	17																		0.16
27-2	Format kvikk 160	73	<10	<10	<10	<10	<10	100																		0.20
28-1	Format vekst 110	52	<10	<10	<10	19	<10	15																		0.08
45-1	Format vekst 110	42	<6		<7	<14	<100	<36	154	<11	<80	<80	<80	<80	<80	<80			<13	<14	<25	<27	<20	31	0.61	
52-1	Opti drektig våt	<14	<6		<7	<14	<100	<36	<15										<13	<14	<25	<27	<20	<11	1.40	
59-1	Opti norm	45	<6		449	<14	<100	<36	529	<11	<80	<80	<80	<80	<80	<80			<13	<14	<25	<27	<20	<11	0.46	
60-1	Natura slaktegris	43	<6		<7	23	<100	<36	<15	<11	<80	<80	<80	<80	<80	127			<13	<14	<25	<27	37	40	2.00	
76-1	Format vekst 110	<14	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	<20	<11	0.06		
86-1	Format vekst 110	<14	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	23	174	46	<20	<11	0.40		
124-1	Format vekst 110	76	<6	<40	51	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	193	46	33	<11	0.07		
126-1	Opti lakta	<14	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	178	46	<20	<11	0.31		
126-2	Opti vital pluss	79	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	90	<27	<20	<11	1.15		
126-3	Opti norm våt	100	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	252	<27	<20	<11	0.73		
136-1	Format kvikk 160	55	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	120	120	<13	<14	129	<27	<20	<11	0.64	
136-2	Formal laktasjon	<14	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	167	<27	<20	<11	1.87		
149-1	Format kvikk 160 smågris	75	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	784	94	185	<11	0.21		
156-1	Øko svin Grøstad	88	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	<14	560	46	82	<11	1.34		
161-1	Format vekst 120	145	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<13	23	554	46	<20	<11	0.01		
169-1	Ideal junior	168	10	<40	<7	<14	<100	219	<15	<11	<50	<80	<80	<80	<80	<80	120	<80	<13	<14	199	46	<20	18	0.90	

**Appendix Table 3.** Concentrations of mycotoxins in individual samples of complete feed for dogs (19 samples) in 2016. All concentrations in µg/kg. DON=deoxynivalenol, 3aDON=3-acetyl-DON, 15aDON=15-acetyl-DON, T2=T-2 toxin, HT2=HT-2 toxin, NIV=nivalenol, ZEA=zearalenone, aZOL=alpha-zearalenol, bZOL=beta-zearalenol, E.nov=ergonovine, E.sin=ergosine, E.tam=ergotamine, E.cor=ergocornine, aE.cry=alpha-ergocryptine, E.cri=ergocristine, FB1=fumonisin B1, FB2= fumonisin B2, EA=enniatiin A, EA1=enniatiin A1, EB=enniatiin B, EB1=enniatiin B1, MON=moniliformin, BUT=5-acetamidebutenolid, CUL=15-hydroxy-culmorin, STE=sterigmatocystin, OTA=ochratoxin A.

Jnr	Type of feed	DON	3a DON	15a DON	T2	HT2	NIV	ZEA	a ZOL	b ZOL	E. nov	E. sin	E. ta	E. cor	aE. cry	E. cri	Fum B1	Fum B2	EA	EA1	EB	EB1	MON	BUT	CUL	STE	OTA
2-1	Diadog puppy	226	<10	<10	<10	<10	<10	22																			0.22
4-1	Fish 4 dogs salmon	19	<10	<10	<10	<10	<10	7																			0.37
5-1	Labb sensitiv	95	<10	19	<10	<10	<10	15																			0.15
18-1	Appetitt senior	73	<10	15	13	10	12	7																			0.10
18-2	Appetitt light	89	<10	17	<10	10	16	17																			0.27
36-1	Acana Wild Prairie	11	<10	<10	<10	<10	<10	<3																			0.24
36-2	Acana Pacifica	27	<10	<10	<10	20	<10	<3																			0.64
37-1	Pedigree mini	60	<10	10	<10	<10	<10	11																			0.11
38-1	Appetitt	75	<10	18	<10	<10	10	9																			0.14
39-1	Labb	240	<10	39	15	21	21	9																			0.03
40-1	Troll E	93	<10	13	<10	18	12	8																			0.67
42-1	Eukanuba	201	<10	28	<10	50	11	23																			0.74
43-1	Ukjent	131	<10	27	<10	45	11	32																			0.06
63-1	Beste venn, voksen hund	23	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<20	<64	<11	<12	0.20
89-1	Godbiten, Norsk d.mat	<14	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<20	<64	<11	<12	0.01
89-2	Fr.tørket vom, Norsk d.mat	<14	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<20	<64	<11	<12	0.01
139-1	VetSolution Hepatic	23	<6	<40	<7	<14	<100	247	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	<20	<64	<11	<12	0.48
148-1	Eukanuba, puppy	<14	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	<25	<27	33	<64	<11	<12	0.12
167-1	Maxdog fullkost	195	<6	<40	<7	<14	<100	<36	<15	<11	<50	<80	<80	<80	<80	<80	<80	<80	<13	<14	41	<27	84	<64	<11	<12	0.31

**Appendix Table 4.** Concentrations of aflatoxin B1, B2, G1, G2 in individual samples of maize (10 samples) in 2016. All concentrations in µg/kg.

Jnr	Afla B1	Afla B2	Afla G1	Afla G2
3-1	<0.25	<0.10	<0.20	<0.15
12-1	<0.25	<0.10	<0.20	<0.15
41-1	<0.25	<0.10	<0.20	<0.15
46-1	<0.25	<0.10	<0.20	<0.15
48-1	<0.25	<0.10	<0.20	<0.15
56-1	<0.25	<0.10	<0.20	<0.15
98-1	<0.25	<0.10	<0.20	<0.15
104-1	<0.25	<0.10	<0.20	<0.15
162-1	0.38	<0.10	<0.20	<0.15
168-1	0.38	<0.10	<0.20	<0.15

**Appendix Table 5.** Concentrations of aflatoxin B1, B2, G1 and G2 in individual samples of complementary feed for ruminants (46 samples) in 2016. All concentrations in µg/kg.

Jnr	Type of feed	Afla B1	Afla B2	Afla G1	Afla G2
8-1	Formel premium 80	<0.25	<0.10	<0.20	<0.15
8-2	Formel favør 80	<0.25	<0.10	<0.20	<0.15
8-3	Formel fullfor	<0.25	<0.10	<0.20	<0.15
9-1	Melketopp nøytral	<0.25	<0.10	<0.20	<0.15
10-1	Drøv kjøttfe	<0.25	<0.10	<0.20	<0.15
11-1	Drøv sau mjølkefor	<0.25	<0.10	<0.20	<0.15
11-2	Drøv geit	<0.25	<0.10	<0.20	<0.15
21-1	Drøv energirik låg	0.38	<0.10	<0.20	<0.15
22-1	Drøv middelslåt	<0.25	<0.10	<0.20	<0.15
23-1	Drøv seinslåt	<0.25	<0.10	<0.20	<0.15
24-1	Formel favør 90	<0.25	<0.10	<0.20	<0.15
24-2	Formel biff	<0.25	<0.10	<0.20	<0.15
28-2	Formel biff intensiv	<0.25	<0.10	<0.20	<0.15
28-3	Formel sau	<0.25	<0.10	<0.20	<0.15
28-4	Formel geit	<0.25	<0.10	<0.20	<0.15
34-1	Formel elite	<0.25	<0.10	<0.20	<0.15
35-1	Storfefor	<0.25	<0.10	<0.20	<0.15
45-2	Formel elite 80	<0.25	<0.10	<0.20	<0.15
50-1	Drøv middelslåt	<0.25	<0.10	<0.20	<0.15
51-1	Drøv energirik	<0.25	<0.10	<0.20	<0.15
53-1	Drøv 12000	0.38	<0.10	<0.20	<0.15
54-1	Formel sau ekstra	0.38	<0.10	<0.20	<0.15
54-2	Formel kalv	<0.25	<0.10	<0.20	<0.15
58-1	Melketopp nøytral	<0.25	<0.10	<0.20	<0.15
58-2	Nor500	<0.25	<0.10	<0.20	<0.15
58-3	Toplac nøytral	<0.25	<0.10	<0.20	<0.15
61-1	Natura drøv 19	<0.25	<0.10	<0.20	<0.15
71-2	Krosset bygg tilsatt maxammon	<0.25	<0.10	<0.20	<0.15
77-1	Natura drøv 19	<0.25	<0.10	<0.20	<0.15
87-1	Formel elite 80	<0.25	<0.10	<0.20	<0.15
111-1	Formel energi basis 80	<0.25	<0.10	<0.20	<0.15
122-1	Drøv middelslåt	<0.25	<0.10	<0.20	<0.15
123-1	Formel elite 80	<0.25	<0.10	<0.20	<0.15
125-1	Melketopp høg	<0.25	<0.10	<0.20	<0.15
125-2	Sau/lam appetitt	<0.25	<0.10	<0.20	<0.15
135-1	Formel elite 80	<0.25	<0.10	<0.20	<0.15
135-2	Formel energi basis 80	<0.25	<0.10	<0.20	<0.15
155-1	Drøv seinslåt	<0.25	<0.10	<0.20	<0.15
158-1	Drøv energi	<0.25	<0.10	<0.20	<0.15
159-1	Formel favør 80	<0.25	<0.10	<0.20	<0.15
159-2	Formel energi pre 70	<0.25	<0.10	<0.20	<0.15
160-1	Formel premium 90	<0.25	<0.10	<0.20	<0.15
160-2	Formel elite 80	<0.25	<0.10	<0.20	<0.15
160-3	Formel biff intensiv	<0.25	<0.10	<0.20	<0.15
179-1	Formel favør 80	<0.25	<0.10	<0.20	<0.15
179-2	Formel sau	<0.25	<0.10	<0.20	<0.15

Appendix Table 6. Concentrations of *Clostridium perfringens*, *Salmonella* sp. and *E. coli* in individual samples (68 samples) of raw food for dogs in 2016.

Jnr.	Name of feed	Prod. country	C.perfringens cfu/g	Salmonella (in 25 g)	E.coli cfu/g
22-48	V&H Active	Norway	<100*	ND	50
22-49	V&H Puppy	Norway	<100*	ND	509
22-50	V&H Active m/laks	Norway	1 000	ND	50
22-482	MUSH BARF Gris	Finland	<10	ND	109
22-503	F&J Slakteavf. av storfe/svin	Norway	<10	ND	10
22-504	F&J Slakteavf. av storfe/svin	Norway	<10	ND	40
22-505	F&J Slakteavf. av storfe/svin	Norway	<10	ND	190
22-506	Hallafoeder Kyckling Eksklusive	Sweden	<10	ND	10
22-507	Hallafoeder Färskfoder Bruks	Sweden	<10	ND	2,000
22-508	Hallafoeder Färskfoderburgare	Sweden	<10	ND	9,000
22-509	Provit Vom og Kjøtt	Norway	<10	ND	10
22-554	Provit Kylling m/vit.	Norway	<10	ND	<10
22-555	MUSH BARF Starter Formula	Finland	<10	ND	10
22-556	MUSH BARF Puppy Formula	Finland	<10	ND	<10
22-557	V&H Vom Active	Norway	<10	ND	40
22-558	V&H Vom Taste	Norway	<10	ND	<10
22-587	KjøttNils, Pølser rått 1kg	Norway	<10	ND	140
22-588	Provit Kylling	Norway	<10	ND	<10
22-589	V&H Active	Norway	20	ND	6,300
22-590	V&H Vom	Norway	120	ND	4,400
22-591	V&H Vom Taste	Norway	<10	ND	<10
22-673	Provit Vom og Lam	Norway	100	ND	<10
22-674	Provit Kylling	Norway	<10	ND	20
22-675	Provit Vom	Norway	<10	ND	<10
22-743	V&H Taste m/kylling	Norway	10	ND	280
22-744	V&H Active m/laks kj.b.	Norway	10	ND	70
22-745	V&H Active m/laks pølse	Norway	100	ND	<10
22-746	V&H Digestive m/ris	Norway	100	ND	309
22-759	DSH, avskjær og skrog fra kalkun	Norway	60	ND	70,000
22-760	V&H Puppy	Norway	600	ND	1,500
22-761	Provit Vom og kjøtt	Norway	500	ND	600
22-810	V&H Taste Lam	Norway	<10	ND	130
22-811	V&H Taste Vom	Norway	<10	ND	<10
22-812	V&H Taste Kylling	Norway	<10	ND	100
22-854	Nortura Slakteavfall	Norway	<10	ND	718
22-855	Nortura Slakteavfall	Norway	<10	ND	581
22-856	Nortura Slakteavfall	Norway	<10	ND	990
22-857	Nortura Slakteavfall	Norway	<10	ND	454
22-858	Nortura Slakteavfall	Norway	<10	ND	681
22-892	Provit Vom, kjøtt, laks	Norway	<10	ND	945
22-893	Provit Vom og lam	Norway	60	ND	536
22-894	Provit Kylling	Norway	<10	ND	90
22-902	Natures Menu, Country Hunter	England	<10	S. Mbandaka	10
22-921	V&H Taste Storfevom	Norway	<10	ND	10
22-922	V&H Taste Kylling	Norway	10	ND	10
22-939	Brit Pr. Sausage Sports Formula	?	<10	ND	<10
22-940	V&H Kylling	Norway	<10	ND	10
22-941	Provit Vom og Lam	Norway	10	ND	20
22-942	V&H Puppy	Norway	160	ND	<10
22-949	V&H Kylling	Norway	30	ND	<10
22-973	Provit	Norway	70	ND	1,400
22-974	V&H med laks	Norway	<10	ND	60
22-975	V&H Puppy	Norway	20	ND	40
22-976	V&H Digestive	Norway	100	ND	473
22-977	V&H Vom Taste Kylling	Norway	40	ND	180
22-991	Provit Kylling m/vit.	Norway	<10	ND	<10
22-992	Provit pølser á 500 g	Norway	70	ND	3,600



22-993	V&H pølse	Norway	10	ND	40
22-1005	Provit Vom	Norway	<10	ND	<10
22-1006	Provit Vom og kjøtt	Norway	40	ND	3,700
22-1007	V&H? Vom Active	Norway	<10	ND	10
22-1008	V&H? Vom Puppy	Norway	10	ND	90
22-1049	V&H Puppy	Norway	<10	ND	845
22-1050	Norsk Dyremat Vom	Norway	10	ND	2,000
22-1052	Provit Vom	Norway	<10	ND	10
22-1053	Provit Vom og lam	Norway	10	ND	420
22-1054	Provit Vom og kjøtt	Norway	60	ND	2,100
22-1055	V&H Active	Norway	20	ND	60

ND = Not Detected

\* Analysed by direct plating on Sheep blood agar.

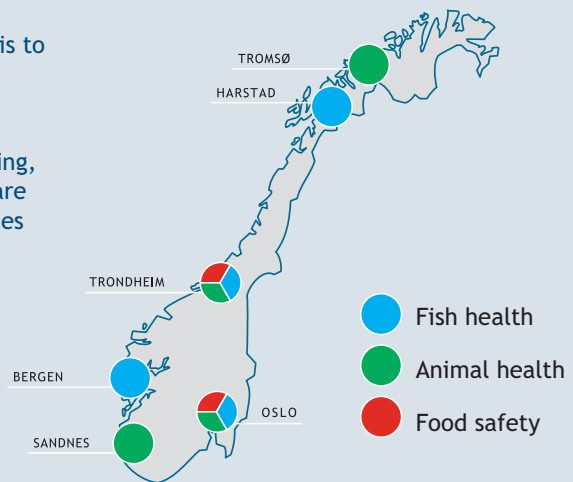
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