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Summary

A single load of corn naturally contaminated with aflatoxin (1,074 ppb), fumonisin (8.3 ppm), and ochratoxin A (206 ppb) was procured from central Oklahoma to evaluate the role of cleaning to remove mycotoxin contamination in corn. Corn was divided into twenty 333-lb lots, which were then cleaned using an EBM Gentle Roll corn cleaner to remove overs (material > 1/2 inches) and unders (material < 3/16 inches). The resultant 4 treatments included: 1) uncleaned corn; 2) overs from cleaned corn; 3) cleaned corn; and 4) unders from cleaned corn. Samples of each fraction were analyzed for mycotoxin content using multiclass liquid chromatography tandem mass spectrometry.

Cleaning generated approximately 6% screenings (unders + overs), and reduced ($P < 0.05$) aflatoxin by an average of 26%. Cleaning also reduced ($P < 0.05$) fumonisin by 45%, but did not impact ochratoxin A. Unders had nearly 4 times the aflatoxin and 7.5 times the fumonisin as the uncleaned corn. In conclusion, cleaning corn may substantially reduce mycotoxin contamination, but the resultant screenings should be used cautiously.

Introduction

Mycotoxins are naturally-produced hazards that result from molds that can grow on cereal grains and other commodities, such as peanuts, cottonseed, and soybeans. The primary species causing mycotoxins are aspergillus (aflatoxin and ochratoxin) and fusarium (fumonisins and zearaloxone) molds. Very small quantities of mycotoxins may cause illness in swine or impact production efficiency.¹

Ideally, exclusion of mycotoxins during the growing or receiving processes would prevent their introduction. Binders have also been demonstrated to potentially increase the level of mycotoxins that can be safely fed to pigs, but are not approved for this purpose

¹ Huff, W. E., L. F. Kubena, R. B. Harvey, and J. A. Doerr. 1988. Mycotoxin interactions in poultry and swine. *J. Anim. Sci.* 66:2351-2355.

by U.S. Food and Drug Administration.^{2,3} Thus, additional mitigation strategies are necessary so pork producers have greater flexibility in grain purchasing.

One potential strategy to reduce mycotoxin contamination is to clean corn. The exposed endosperm in broken kernels may be a substrate for additional mycotoxin production, so removing them may reduce the quantity of mycotoxin in the cleaned corn. Furthermore, the physical abrasion from screening may reduce contamination located on the outside of kernels. However, there is limited research about the quantity of mycotoxin reduction due to screening in corn. Therefore, the objective of this study was to quantify the magnitude of mycotoxin contamination that may be removed by cleaning corn.

Procedures

The Kansas State University Institutional Biosafety Committee approved the protocol used in this experiment. A load of corn naturally contaminated with mycotoxins and originating from a single field in central Oklahoma was procured and transported with FDA³ approval to the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Corn was stored for approximately 5 months in a segregated storage bin until used for the experiment.

A total of 6,660 lb of corn was utilized in the experiment. Corn was segregated into twenty 333-lb lots and cleaned by sifting across one or two square-hole screens (1/2 inch and 3/16 inch) using an EBM Gentle Roll screener. Screens were sanitized between lots to prevent lot-to-lot cross-contamination. Neither the order of screening, nor the number of times across the screen impacted ($P > 0.05$) quantity of mycotoxin removed, so those factors were removed from the statistical model. The resultant 4 treatments included: 1) uncleaned corn; 2) overs from cleaned corn; 3) cleaned corn; and 4) unders from cleaned corn. Three 11-lb samples of the uncleaned corn was collected from each lot according to methods described in the 2014 AAFCO Feed Inspector's Manual.⁴ Only 19 of the lots resulted in overs after screening, and the entire quantity of overs in each lot were weighed and retained as samples. The quantity of cleaned corn was weighed, and thirty 11-lb samples collected. All lots resulted in unders, and the entire quantity was weighed and retained as samples.

All samples of the 4 treatments were ground to less than 400 μm using a laboratory-scale Bliss Industries hammermill and 15-gallon commercial vacuum to collect the ground sample. The hammermill and vacuum hose were sanitized between samples, and a new bag used for each sample, to prevent sample-to-sample cross-contamination. Samples were riffle-divided to create sub-samples for lateral flow quick test.

The resultant 139 sub-samples for lateral flow quick test analysis were analyzed for aflatoxin using a Neogen AccuScan Gold reader and Neogen Reveal Q+ Max test strips

² Jacela J. Y. J. M. DeRouche, M. D. Tokach, R. D. Goodband, J. L. Nelssen, D. G. Renter, and S. S. Dritz. 2010. Feed additives for swine: Fact sheets - flavors and mold inhibitors, mycotoxin binders, and antioxidants. J. Swine Health Prod. 18:27-32.

³ U.S. Food & Drug Administration. Action levels for aflatoxins in animal feeds. 1994. Compliance Policy Guide Sec. 683.100.

⁴ Association of American Feed Control Officials. Feed inspector's manual, 2014.

by a single technician. A total of 36 composite samples across the 4 treatments were created using riffle division and analyzed for multiple mycotoxins via multiclass liquid chromatography tandem mass spectrometry (LC-MS/MS) at the North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND.

Data were analyzed using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

Results and Discussion

Cleaning generated 0.06% overs and 5.86% unders by weight (Table 1). The uncleaned corn contained 1,074 ppb aflatoxin, which was primarily aflatoxin B₁ and minimal aflatoxin B₂. This is substantially greater than the maximum limit allowed for corn to be used in swine diets, which ranges from 20 to 200 ppb total aflatoxin, depending upon the phase of production.³ The uncleaned corn also had detectable levels of fumonisin and ochratoxin A, but at levels below those known to impact swine health or production.

Treatments developed from cleaning had different ($P < 0.0001$) total aflatoxin, aflatoxin B₁, aflatoxin B₂, total fumonisin, fumonisin B₁, fumonisin B₂, and ochratoxin A concentrations (Table 1). Overs had lower ($P < 0.050$) concentrations of total aflatoxin, B₁, and B₂, but higher ($P < 0.050$) concentrations of total fumonisin, B₁, and B₂ compared to uncleaned corn. There were similar ($P > 0.050$) levels of ochratoxin A between overs and uncleaned corn. While trichothecene (T-2) and sterigmatocystin were undetected in the uncleaned corn, there were detectable levels in overs.

Cleaned corn had 26% less total aflatoxin ($P < 0.050$; 789 vs. 1,074 ppb, respectively) and 45% less total fumonisin ($P < 0.050$; 4.5 vs. 8.3 ppm, respectively) than uncleaned corn. As expected, there was substantial lot-to-lot variation in analyzed total aflatoxin (Figure 1) and total fumonisin (Figure 2), both before and after cleaning. Still, cleaning consistently reduced ($P < 0.050$) total aflatoxin in all except one lot, and reduced ($P < 0.050$) total fumonisin in all lots. Cleaning did not impact ($P > 0.050$) ochratoxin A concentration in uncleaned vs. cleaned corn ($P > 0.050$; 198 vs. 206 ppb, respectively). The ability of cleaning to reduce contamination of some mycotoxins, but not others, may be due to the location of the type of mycotoxin on the kernel itself, or how the toxins attach differently to kernel structures. Further research is needed to better understand this phenomenon.

Unders had nearly 4 times greater total aflatoxin ($P < 0.050$; 4,224 vs. 1,074 ppb), 7.5 times greater total fumonisin ($P < 0.050$; 3,976 vs. 1,005), and 2.7 times greater ochratoxin A ($P < 0.050$; 562 vs. 206 ppb) as the uncleaned corn. This concentration of mycotoxin in the screenings (overs + unders) fraction is notable and can have both positive and negative implications. If feeding whole corn contaminated with aflatoxins or fumonisins, these data suggest that cleaning is an effective method to legally reduce mycotoxin contamination and render the product safer for animal consumption. These data suggest that cleaning prior to storage may be an important step to reduce the overall mycotoxin contamination and potential for proliferation during the storage period if storing whole corn contaminated with aflatoxins or fumonisins. However, care needs to

be taken with the resultant screenings. It is common practice to feed screenings, either as a distinct commodity or by addition to the ground corn bin, which may lead to high risk pulses of mycotoxins in swine feed.

Finally, the accuracy of the quick test analysis was compared to paired sub-samples analyzed by LC-MS/MS. The quick test, which is validated to a maximum aflatoxin level of 150 ppb, had variable results within sample (Figure 3). Across multiple samples, however, the quick test method was accurate in both uncleaned and cleaned corn. The overall mean aflatoxin level predicted by the quick test was similar to overall levels from the LC-MS/MS method ($P > 0.050$; 960 vs. 1,074 in uncleaned and 732 vs. 789 ppb in cleaned corn). However, the quick test method underestimated aflatoxin in both overs and unders ($P < 0.050$; 82 vs. 298 ppb in overs and 2,870 vs. 4,224 ppb in unders). This variability in the quick test, especially in unders, is likely due to the multiple dilutions necessary to estimate high aflatoxin levels in the quick test method beyond 150 ppb, as each dilution introduces additional error potential.

In conclusion, cleaning corn may be one method to reduce aflatoxin and fumonisin. A limitation of this experiment is that a single supply of corn was utilized, so it is still unknown how these results may extend to corn with less contamination. While it is common for the feed industry to utilize screenings, these results suggest that screenings may have heightened risk for mycotoxins. In years of high mycotoxin grain, it may be necessary to divert screenings to species or phases of production that are less susceptible to mycotoxicosis.

Table 1. Effects of cleaning corn on mycotoxin concentration¹

Item	Uncleaned corn	Screened fraction		SEM	P =	
		Overs	Cleaned corn			Unders
Percentage of weight, %	100	0.06	94.1	5.86	-	
LC-MS/MS analysis						
<i>n</i>	15	3	15	3	-	-
Aflatoxin (total), ppb	1,074 ^b	298 ^d	789 ^c	4,224 ^a	70.2	< 0.0001
B ₁ , ppb	1,005 ^b	258 ^d	733 ^c	3,976 ^a	66.1	< 0.0001
B ₂ , ppb	69.3 ^b	40.0 ^c	56.4 ^c	248 ^a	7.46	< 0.0001
G ₁ , ppb	< 20	< 20	< 20	< 20	-	-
G ₂ , ppb	< 20	< 20	< 20	< 20	-	-
Deoxynivalenol (DON), ppb	< 200	< 200	< 200	< 200	-	-
Fumonisin (total), ppm	8.26 ^c	15.6 ^b	4.50 ^d	60.4 ^a	0.59	< 0.0001
B ₁ , ppm	6.88 ^c	13.2 ^b	3.72 ^d	51.1 ^a	0.38	< 0.0001
B ₂ , ppm	1.38 ^c	2.43 ^b	0.74 ^d	9.32 ^a	0.08	< 0.0001
Trichothecene (HT-2), ppb	< 200	< 200	< 200	< 200	-	-
Trichothecene (T-2), ppb	< 20	34.0	< 20	< 20	-	-
Ochratoxin A, ppb	206 ^b	236 ^b	198 ^b	562 ^a	32.9	< 0.0001
Sterigmatocystin, ppb	< 20	30.0	< 20	< 20	-	-
Zearalenone, ppb	< 50	< 50	< 50	< 50	-	-
Quick test analysis						
<i>n</i>	60	19	30	30	-	-
Aflatoxin (total), ppb	960 ^b	82.0 ^d	732 ^c	2,870 ^a	74.5	< 0.0001

¹ A total of twenty lots of the corn was cleaned across two different screens to isolate clean corn, overs (material > 1/2 inches), and screenings (material < 3/16 inches). Three 11-lb samples of the uncleaned corn, as well as samples from each of the 3 final fractions were collected from each lot, ground and analyzed for mycotoxin concentration.

^{abcd} Means within a row with different superscripts differ $P < 0.05$.

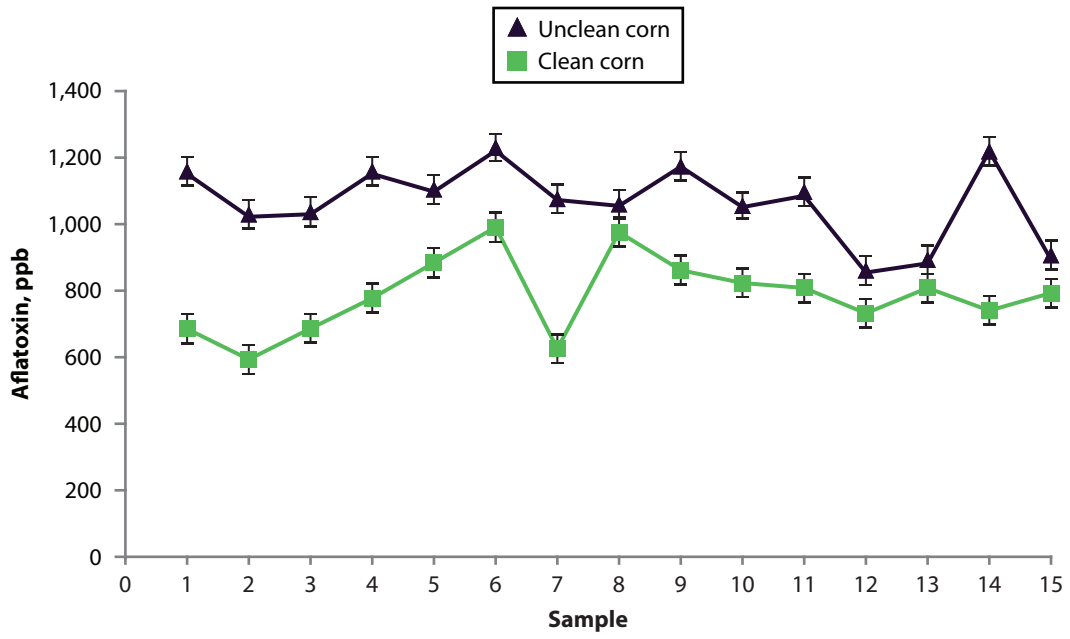


Figure 1. Aflatoxin level in corn before and after cleaning.

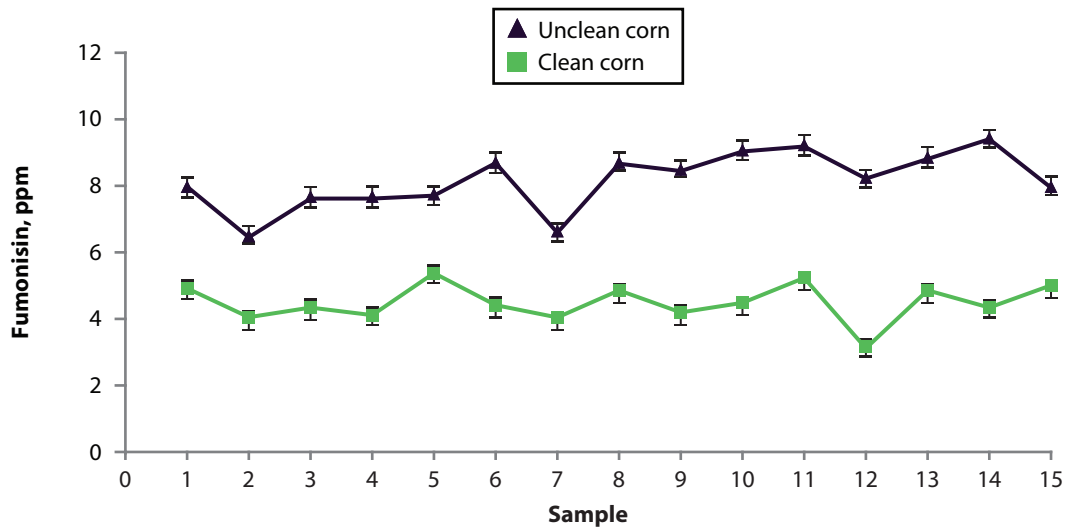


Figure 2. Fumonisin level in corn before and after cleaning.

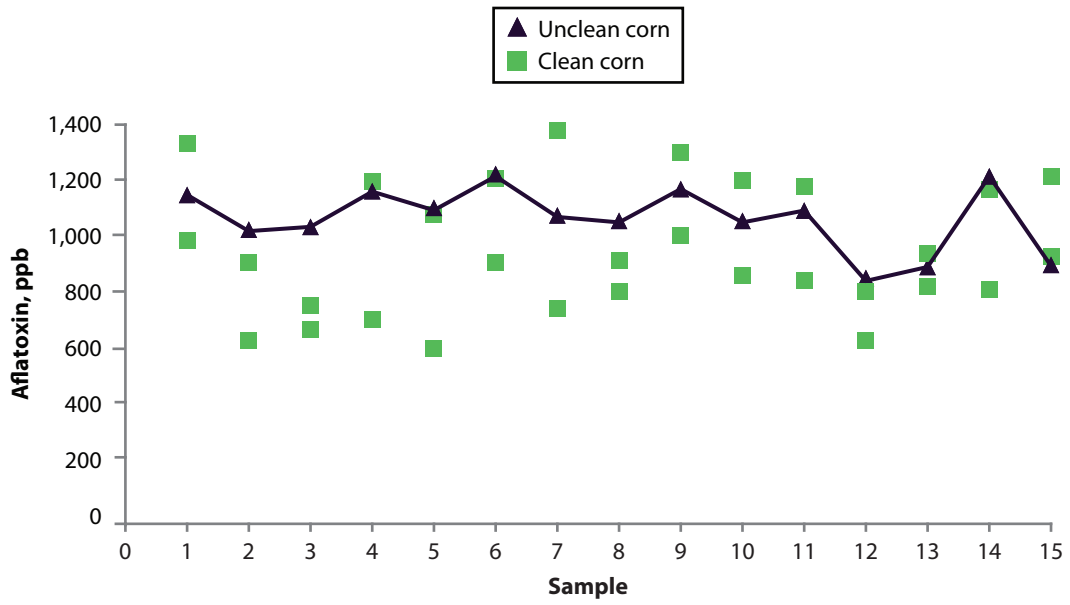


Figure 3. Aflatoxin level estimated by quick test vs. LC-MS/MS in uncleaned corn.