Effect of cleaning corn on mycotoxin concentration and nursery pig growth performance

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ABSTRACT: Mycotoxins are naturally produced hazards that result from molds grown on cereal grains and other commodities. These molds may produce carcinogenic mycotoxins, which can be harmful to humans and animals. Removing broken kernels has been demonstrated to reduce mycotoxin concentration, but with high variability. Therefore, two experiments were conducted to quantify the magnitude of natural mycotoxin concentration that may be reduced by cleaning corn. Two loads of corn that were naturally contaminated with mycotoxins were procured. Corn for Experiment 1 was contaminated with aflatoxin (1,074 parts per billion; ppb), fumonisin (8.3 parts per million; ppm), and ochratoxin A (206 ppb), while corn for Experiment 2 was contaminated with only fumonisin (5.5 ppm). Corn was cleaned by mechanical sieving. For each experiment, corn was divided into twenty 150 kg runs. Runs were randomly assigned to 1 of 4 experimental treatments: 1) no screen 2) 12.7 mm screen, 3) 4.8 mm screen, and 4) 12.7 + 4.8-mm screen. The corn cleaner was sanitized between runs. Three 5 kg corn samples were collected from each run, and analyzed for mycotoxin concentration. In Experiment 1, cleaning reduced ($P < 0.05$) aflatoxin and fumonisin concentration by an average of 26% and 45%, respectively, compared to the original uncleaned corn level, but did not impact ($P > 0.10$) ochratoxin A. The resultant screenings had nearly four times the aflatoxin (4,224 ppb) and 7.5 times the fumonisin concentration (60.4 ppm) as the uncleaned corn. In Experiment 2, cleaning reduced ($P < 0.05$) fumonisin concentration by 32%. The resultant screenings had 19.6 times the fumonisin concentration (65.4 ppm) as the uncleaned corn. To determine the effect that cleaning corn may have on nursery pig growth performance, 360 nursery pigs were used in Experiment 3 to evaluate the impact of cleaning or pelleting on growth performance. Treatments were arranged in a 2 × 3 factorial with corn type (uncleaned vs. cleaned) and feed form (mash vs. pelleted from either mill A or B). Neither cleaning corn nor pellet mill type affected ($P > 0.19$) nursery pig growth performance. Pelleting improved ($P < 0.0001$) gain to feed ratio (G:F) by 7.6% compared to mash diets. These data suggest that cleaning is an effective method to legally reduce aflatoxin and fumonisin concentration, but does not impact animal growth performance. Screenings should be used cautiously when feeding to animals.

Key words: corn, feed, mycotoxin, nursery pig, screenings

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INTRODUCTION

Mycotoxins are fungal secondary metabolites from molds grown on cereal grains and other commodities, such as peanuts, cottonseed, and soybeans. These harmful metabolites can be produced by *Aspergillus, Fusarium* and *Penicillium* fungi. (Rodríguez *et al.*, 2013). These genera can produce aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, T-2 toxin, and zearalenone. Aflatoxins and fumonisins are of high concern because they produce carcinogens and induce disease that can be harmful to both humans and animals (Gelderblom *et al.*, 1988; Huff *et al.*, 1988; Eaton and Gallagher, 1994).

Mycotoxins are often concentrated on the outer seed coat of grains or in dust and broken kernels, which are more susceptible to fungal infection and toxin contamination (Smith and Henderson, 1991). The cracked or broken kernels may result from drought stress or insect damage, and expose nutrients that facilitate fungal growth (Magan and Olsen, 2004).

The amount of mycotoxin contamination in the corn may be reduced by mechanical processing. Screening is a mechanical process in which the grain passes over a screen that contains uniform openings to separate the desired particle size from the original sample. Screenings may include light and broken grains and agricultural seeds, weed seeds, hulls, chaff, joints, straw, elevator or mill dust, sand, and dirt (Association of American Feed Controls Officials, 2018). Screening can remove broken corn and dust to reduce the mean mycotoxin concentration, but with variable success (Smith and Henderson, 1991). Field reports suggest that cleaning corn can substantially reduce mycotoxin concentrations; however, in the literature this has largely been evaluated in the context of artificially inoculated grain. There are limited data demonstrating how screenings affect the mycotoxin concentration in naturally contaminated corn, which could be very different from artificial inoculation, or how removing the screenings may affect the nutrient density and pellet quality. Additionally, it is not known if the reduction in mycotoxin concentrations that result from screening corn is great enough in magnitude to impact swine growth performance. Therefore, the objectives of this experiment were to evaluate the effect of physically cleaning corn naturally contaminated with mycotoxins on the resulting level of contamination, and to evaluate the effects of cleaning on the nutrient density, pellet quality, and the growth performance of nursery pigs fed mash or pelleted diets.

MATERIALS AND METHODS

General

The Kansas State University Institutional Biosafety Committee and Institutional Animal Care and Use Committee approved the procedures used in this experiment (#1178 and 3529, respectively). Aflatoxin is a biosafety level-2 pathogen, and cleaning was conducted under containment conditions.

Experiment 1

A total of 3,000 kg of corn that was naturally contaminated with mycotoxins was procured from a single field in central Oklahoma and transported with Food and Drug Administration (FDA) approval to the Kansas State University Cargill Feed Safety Research Center. The dryland corn was a drought tolerant hybrid (6355 Dekalb) with 30-inch row spacing, in no-till sandy loam soil, that contained adequate nutrition by analysis. Glyphosate and atrazine were applied to reduce weed occurrence. The crop experienced extremely low rainfall in May, slightly higher temperature and rainfall in July, and substantially greater humidity in July, August, and September compared to the 5-year average, and was harvested after 158 days (Table 1).

The 3,000 kg of uncleaned corn was divided into twenty 150 kg runs, with run as the experimental unit. A single run consisted of three 50 kg barrels. A total of sixty 50 kg barrels were filled from the original 3,000 kg. To account for the segregation of broken kernels and foreign material while unloading product from the bin, three sets of 20 barrels were filled (1–20, 21–40, and 41–60). Each barrel within each set of 20 were randomized to run number 1 through 20, for a total of 3 barrels per run. Screens were cleaned by aspiration between each run to prevent cross-contamination. Corn was cleaned using a commercial corn cleaner (EBM Gentle Roll, model# 24S-1-HB-F-FF, Norfolk, NE), with resultant products being corn + thrus and overs, corn + overs and thrus, and cleaned corn (Figure 1). Runs were cleaned using 1 of 4 experimental treatments: 1) no screening (control), 2) 12.7 mm screen, 3) 4.8 mm screen, and 4) 12.7 + 4.8 mm screen. Treatments 1, 2, 3, and 4 had 20, 10, 10, and 10 replications, respectively. Three samples of the resultant product of each run were collected by probing according to the Association of American Feed Controls Officials Feed Inspector's Manual (2014).

Table 1. Oklahoma agronomic climate conditions[1]

	Month					
Item	April	May	June	July	August	September
2016						
Temperature, °C	17.2	19.6	26.1	28.2	27.4	25.3
Rainfall[2], cm	0.66	0.28	0.25	0.53	0.04	0.11
Humidity, %	65.6	74.6	68.0	68.7	67.8	69.6
5-year average (2012–2016)						
Temperature, °C	16.2	20.3	25.9	27.8	27.3	24.4
Rainfall[2], cm	0.41	0.62	0.31	0.45	0.16	0.16
Humidity, %	66.7	70.8	67.3	63.7	61.5	63.2

[1]Data collected from Mesonet database; Norman, OK site.

[2]Average daily rainfall.

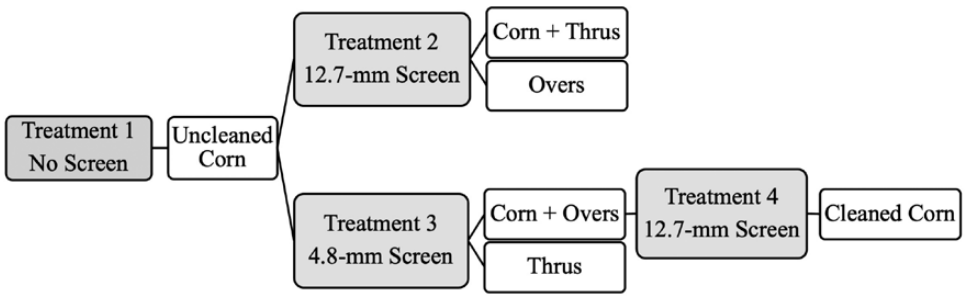

Figure 1. Experiment 1 Treatments.

Experiment 2

A total of 3,000 kg corn that was naturally contaminated with mycotoxins was procured from a single field in Southeast Kansas and transported to the Kansas State University Cargill Feed Safety Research Center. The irrigated corn with 30-inch row spacing was a herbicide tolerant kernel with Glyphosate traits planted in soil that contained medium to high amounts of phosphorus pentoxide and potassium oxide by analysis. Corn kernels also contained insect-resistant technology and crop rotation practices were used to reduce pest occurrence. Conventional tillage practices and storage bin aeration were performed. The crop experienced slightly less rainfall in May and June, and substantially more rainfall and humidity in July and August compared to the 5-year average (Table 2). The uncleaned corn was again divided into twenty 150 kg runs, with run as the experimental unit. Treatments and procedures for cleaning and sampling were as described in Experiment 1.

Sample Preparation

Resultant samples were ground to ≤ 400 µm using a laboratory-scale hammermill (Bliss Industries, LLC, Model Eliminator, Ponca City, OK) equipped with a 56-liter commercial vacuum to mimic air-assist and collect the ground sample. To reduce cross-contamination, the hammermill and vacuum hose were cleaned by aspiration between samples, and a new vacuum bag was used for each sample.

Mycotoxin Analyses

Samples were analyzed for mycotoxin concentrations using a rapid analysis and LC–MC/MS. The rapid analysis was done to determine if cleaning impacted total mycotoxin concentrations in a manner similar to that of a production environment. The LC–MS/MS analysis was done to determine if cleaning impacted the specific fractions of each mycotoxin of interest. All samples were analyzed by a single technician. Total aflatoxin and fumonisin concentration were determined by lateral flow rapid technique analysis using an AccuScan Gold scanner with Reveal Q+ Max (limit of detection = 3 ppb; Neogen Corporation, Lansing, MI) aflatoxin test strips and Reveal Q+ fumonisin (limit of detection = 0.3 ppm) test strips for experiments 1 and 2, respectively. When sample concentration exceeded the maximum test strip threshold, samples were diluted with distilled water or a 65% ethanol solution per manufacturer recommendations. After rapid technique analysis, sub-samples were riffle divided into composite samples and analyzed for

Table 2. Kansas agronomic climate conditions[1]

	Month						
Item	March	April	May	June	July	August	September
2016							
Temperature, °C	11.5	15.6	18.0	26.1	27.1	26.2	22.8
Rainfall[2], cm	0.23	0.50	0.25	0.17	0.44	0.46	0.27
Humidity, %	55.9	58.9	70.1	67.4	72.3	70.5	69.0
5-year average (2012–2016)							
Temperature, °C	10.0	14.9	19.4	25.0	26.8	25.9	22.6
Rainfall[2], cm	0.18	0.35	0.34	0.23	0.24	0.23	0.20
Humidity, %	59.2	60.4	68.1	67.8	65.2	64.6	66.2

[1]Data collected from Mesonet database; Sedan site.

[2]Average daily rainfall.

multiple mycotoxins by multiclass liquid chromatography tandem mass spectrometry (LC–MS/MS) at the North Dakota State University Veterinary Diagnostic Laboratory in Fargo. Sample loss throughout the mycotoxin analysis resulted in lower sample sizes for some analyses.

Experiment 3

The trial was conducted at the Kansas State University Segregated Early Weaning Facility. All diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center.

A total of 360 nursery pigs (initially 8.8 kg BW) were utilized in a 28-d experiment. There were five pigs per pen and 12 pens per treatment. Upon arrival to the barn, pigs were allotted in a completely randomized design. A common starter diet was fed for 10 d post-weaning to allow the pigs to acclimate to the facility. On d 10, pens were allotted to 1 of 6 dietary treatments. Treatments were arranged in 2 × 3 factorial arrangement with corn type (uncleaned vs. cleaned corn that was naturally contaminated with fumonisin from Experiment 2) and feed form (mash vs. pelleted from either mill A or B). The same formula was used in all treatments (Table 3). Diets were pelleted on either pellet mill A (master model 1000 HD, California Pellet Mill Co., Crawfordsville, IN) or pellet mill B (model 3016-4, California Pellet Mill Co., Crawfordsville, IN). While pellet mill types differed, the conditioning temperature (85°C ± 1°C), conditioner retention time (30 s), pellet die size (4.0 × 22-mm), and production rate percentage (60%) based on pellet mill capacity were held constant. Complete diets were analyzed for proximate analysis at Ward Laboratories Inc. using AOAC Methods 935.29, 990.03, 978.10, 920.39, and 942.05 for dry matter, crude protein, crude fiber, ether extract, and ash, respectively (Table 4). Furthermore, pelleted diets were analyzed for pellet durability index using the Holmen NHP100 (TekPro Ltd., Norfolk, United Kingdom) for a 60 s test time, and pellet fines percentage by sifting pellets across a U.S. #6 sieve to determine the quantity of fines. Pigs and feeders were weighed on d 0, 7, 14, 21, and 28 of the trial to determine average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F).

Statistical Analyses

Data were analyzed using the GLIMMIX and CORR procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC) with the Kenward–Roger adjustment. In Experiment 1 and 2, run was the experimental unit, while pen was the experimental unit for Experiment 3. In Experiment 1 and 2, the average range between values for LC–MS/MS were compared using Tukey's studentized range for pairwise analysis. In Experiment 3, pre-planned contrast statements of uncleaned vs. cleaned corn, pellet mill type A vs. pellet mill type B, and mash vs. pelleted diets were conducted using the CONTRAST statement of SAS. Results for treatment criteria were considered significant at $P \leq 0.05$ and marginally significant if $0.05 < P \leq 0.10$.

RESULTS

Experiment 1

Screening impacted ($P < 0.05$) the concentration of all detectable mycotoxins in corn except Ochratoxin A ($P = 0.45$; Table 5). The reduction in mycotoxin concentration was due to the 4.8 mm screen. A single pass across the 4.8 mm screen reduced ($P < 0.05$) contamination in all measured mycotoxins except Ochratoxin A. Notably, cleaning corn with only a 4.8 mm screen reduced a substantial

Table 3. Composition of diets, Experiment 3

Ingredient	%
Ground corn	64.2
Soybean meal, 46.5% CP	29.9
Monocalcium phosphate, 21% P	1.35
Limestone	0.85
Salt	0.75
L-Lysine HCl	0.50
DL-Methionine	0.21
L-Threonine	0.21
L-Tryptophan	0.03
L-Valine	0.13
Trace mineral premix[1]	0.15
Vitamin premix[2]	0.25
Phytase[3]	0.015
Choice White Grease	1.50
Total	100.00
Calculated analysis	
Standardized ileal digestibility (SID) amino acids	%
Lysine	1.30
Isoleucine:Lysine	55
Leucine:Lysine	114
Methionine:Lysine	37
Met and Cystine:Lysine	58
Threonine:Lysine	63
Tryptophan:Lysine	18.5
Valine:Lysine	70
ME, kcal/kg	687
CP, %	20.4
SID lysine:metabolizable energy, g/Mcal	3.90
Total lysine, %	1.44
Calcium, %	0.69
Phosphorus, %	0.67
Available Phosphorus, %	0.46
Fat, %	4.1

[1]Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.

[2]Provided per kilogram of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.

[3]HiPhos 2700 (DSM, Het Overloon, Netherlands) provided 476 phytase units (FTU)/kg of diet, for an estimated release of 0.10% available P.

magnitude of contamination: a 36% reduction on aflatoxin and a 45% reduction in fumonisin compared to the uncleaned corn. Screening across the 12.7 mm screen alone did not impact ($P > 0.05$) corn mycotoxin concentration, and did not further reduce ($P > 0.05$) contamination when used in combination with the 4.8 mm screen. After cleaning corn across both the 4.8 and 12.7 mm screen, aflatoxin and fumonisin were reduced by 26% and 45%, respectively. The difference between the 4.8 mm screen and a combination of cleaning with a 4.8 and 12.7 mm screen in aflatoxin concentration reduction is likely due to analytical variability. The rapid technique method only analyzed total aflatoxin concentration, with similar reduction ($P < 0.05$) when screened with 4.8 mm screen, but no impact ($P > 0.05$) of the 12.7 mm screen.

To determine the mycotoxin concentration of each fraction in the uncleaned corn, fractions were separated and analyzed (Table 6). These individual fractions included: 1) overs, or material that did not pass through the 12.7 mm screen, 2) cleaned corn, or material that passed through the 12.7 mm screen, but not the 4.8 mm screen, and 3) thrus, or material that passed through both the 12.7 and 4.8 mm screens. Overs, typically including pieces of corn cob or husk, accounted for only 0.06% of the weight of end product. Cleaned corn was 94.1% of the initial weight, with thrus, typically including broken kernels and dust, representing 5.86% of the initial weight of the uncleaned corn.

Physical cleaning impacted ($P < 0.05$) mycotoxin concentration in all fractions. Material caught by the 12.7 mm screen, or overs, had lower ($P < 0.05$) concentrations of total aflatoxin, but greater ($P < 0.05$) total fumonisin, compared to cleaned corn. There was no difference ($P > 0.05$) in ochratoxin A concentration between overs and cleaned corn. Interestingly, there were detectable levels for trichothecene (T-2) and sterigmatocystin (34.0 vs. 30.0, respectively) in the overs, despite being undetected in the cleaned corn.

As expected, material passing through both the 12.7 and 4.8 mm screens had greater ($P < 0.05$) aflatoxin mycotoxin concentrations compared to cleaned corn. The thrus had 5.3 times greater aflatoxin, 13.4 times greater fumonisin, and 2.8 times greater ochratoxin A than cleaned corn. The rapid technique method again only analyzed total aflatoxin concentration, with similar pattern for aflatoxin reduction ($P < 0.05$), but the means for the cleaned corn samples were more consistent with those measured by LC–MS/MS than means for overs or thrus.

Experiment 2

Cleaning corn reduced ($P < 0.05$) the concentration of fumonisin; however, unlike experiment 1, fumonisin was the only detectable mycotoxin in this specific source of corn (Table 7). Similar to Experiment 1, the reduction in fumonisin concentration was caused by removing material with a 4.8 mm screen. Notably, this led to a 32% reduction in fumonisin compared to the uncleaned corn.

Again, cleaning corn across the 12.7 mm screen alone did not impact ($P > 0.05$) fumonisin concentration, and did not further reduce ($P > 0.05$) contamination when used in combination with the 4.8 mm screen. After cleaning corn across both the 4.8 and 12.7 mm screen, fumonisin were reduced by 40%. The rapid technique method only analyzed total fumonisin concentration, with similar reduction ($P < 0.05$) when screened with 4.8 mm screen, but no impact ($P > 0.05$) of the 12.7 mm screen.

Again, to determine the fumonisin concentration of each fraction in the uncleaned corn, fractions were separated and analyzed (Table 8). These fractions included: 1) overs, or material that did not pass through the 12.7 mm screen (0.02% by weight); 2) cleaned corn, or material that passed through the 12.7 mm screen, but not the 4.8 mm screen (97.76% by weight); and 3) thrus, or material that passed through both the 12.7 and 4.8 mm screens (2.22% by weight).

Table 4. Chemical analysis of diets (as-fed basis), Experiment 3

Item, %	Mash – Uncleaned[1]	Mash – Cleaned[2]	Type A[3] – Uncleaned	Type A – Cleaned	Type B[4] – Uncleaned	Type B – Cleaned
DM	90.3	90.0	91.0	90.8	91.0	91.2
CP	21.1	20.2	20.7	21.0	21.0	20.8
Crude fiber	2.9	3.3	3.3	3.5	3.5	3.7
Ether extract	3.1	3.9	3.8	3.7	3.3	4.0
Ash	5.7	5.5	5.2	5.3	5.2	5.4

[1]Uncleaned corn contained broken kernels and foreign material.

[2]Cleaned corn had broken kernels < 4.8 mm and foreign material > 12.7 mm removed.

[3]Type A (master model 1000 HD, California Pellet Mill Co., Crawfordsville, IN).

[4]Type B (model 3016-4, California Pellet Mill Co., Crawfordsville, IN).

Table 5. Effect of physical cleaning corn and cleaner screen size on corn mycotoxin concentration[1]

	Screen size (mm) used to clean corn					
Item	Uncleaned Corn	12.7[2]	4.8[3]	12.7 + 4.8[4]	Pooled SEM	P
LC–MS/MS analysis						
n	15	3	3	10	–	–
Aflatoxin (total), ppb	1,074[a]	968[ab]	690[c]	789[bc]	62.3	<0.0001
B_1, ppb	1,005[a]	902[ab]	641[c]	733[bc]	59.4	<0.0001
B_2, ppb	69.3[a]	65.7[ab]	49.0[c]	56.4[bc]	4.07	<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[a]	8.14[a]	4.60[b]	4.46[b]	0.41	<0.0001
B_1, ppm	6.88[a]	6.94[a]	3.89[b]	3.72[b]	0.36	<0.0001
B_2, ppm	1.38[a]	1.20[a]	0.71[b]	0.74[b]	0.06	<0.0001
Trichothecene (HT-2), ppb	<200	<200	<200	<200	–	–
Trichothecene (T-2), ppb	<20	<20	<20	<20	–	–
Ochratoxin A, ppb	206	227	186	198	19.6	0.453
Sterigmatocystin, ppb	<20	<20	<20	<20	–	–
Zearalenone, ppb	<50	<50	<50	<50	–	–
Rapid technique analysis						
n	20	10	10	10	–	–
Aflatoxin (total), ppb	976[a]	872[ab]	693[c]	732[bc]	46.8	<0.0001

[1]A single load of naturally contaminated corn was cleaned by mechanical sieving. Corn was divided into twenty 150 kg runs. Run were randomly assigned experimental treatments and the corn cleaner was sanitized between run. Three 5 kg corn samples were collected from each run, ground, split, and analyzed for mycotoxin concentration by either LC–MS/MS or rapid technique analysis.

[2]Values represent material passing through the 12.7 mm screen.

[3]Values represent material not passing through the 4.8 mm screen.

[4]Values represent material not passing through the 4.8 mm screen, but passing through the 12.7 mm screen.

[a-c]Means within a row with different superscripts differ $P < 0.05$.

Table 6. Effect of fraction type on mycotoxin concentration[1]

	Fraction Type				
Item	Overs[2]	Cleaned corn[3]	Thrus[4]	SEM	P
Percentage of weight, %	0.06	94.1	5.86		
LC–MS/MS analysis					
n	3	10	3		
Aflatoxin (total), ppb	298[c]	789[b]	4,224[a]	79.7	<0.0001
B_1, ppb	258[c]	733[b]	3,976[a]	74.8	<0.0001
B_2, ppb	40.0[b]	56.4[b]	248[a]	7.41	<0.0001
G_1, ppb	<20	<20	<20	–	–
G_2, ppb	<20	<20	<20	–	–
Deoxynivalenol (DON), ppb	<200	<200	<200	–	–
Fumonisin (total), ppm	15.6[b]	4.50[c]	60.4[a]	0.70	<0.0001
B_1, ppm	13.2[b]	3.72[c]	51.1[a]	0.62	<0.0001
B_2, ppm	2.43[b]	0.74[c]	9.32[a]	0.94	<0.0001
Trichothecene (HT-2), ppb	<200	<200	<200	–	–
Trichothecene (T-2), ppb	34.0	<20	<20	–	–
Ochratoxin A, ppb	236[b]	198[b]	562[a]	42.9	<0.0001
Sterigmatocystin, ppb	30.0	<20	<20	–	–
Zearalenone, ppb	<50	<50	<50	–	–
Rapid technique analysis					
n	10	10	10	–	–
Aflatoxin (total), ppb	121[c]	732[b]	2,865[a]	43.5	<0.0001

[1]A 5 kg sample were collected from each run, ground and analyzed for mycotoxin concentration.

[2]Product that did not pass through a 12.7 mm screen.

[3]Product that passed through a 12.7 mm screen but not a 4.8 mm screen.

[4]Product that passed through a 4.8 mm screen.

[abc]Means within a row with different superscripts differ $P < 0.05$.

Physical cleaning impacted ($P < 0.05$) mycotoxin concentration in all fractions. Material caught by the 12.7 mm screen, or overs, had 6 times greater ($P < 0.05$) fumonisin levels compared to cleaned corn. Material passing through both the 12.7 and 4.8 mm screens had nearly 20 times greater ($P < 0.05$) fumonisin than cleaned corn. There was not sufficient sample to analyze the overs using the rapid technique method, but the thrus had over 27 times greater ($P < 0.05$) the fumonisin level of cleaned corn, with means that were numerically similar to those measured by LC–MS/MS.

Experiment 3

To understand the potential impact of corn cleaning on nursery pig performance, corn from Experiment 2 was used in a swine growth experiment. The uncleaned corn had 5.48 ppb fumonisin, while the cleaned corn (material that passed through the 12.7 mm screen, but not the 4.8 mm screen) had 3.33 ppb fumonisin. Diets were then fed as mash, or pelleted in one of two pellet mills, to determine if the inclusion of overs and thrus potentially impacted pellet quality that could be discerned through different pellet mills. As described in Table 4, there was limited impact of cleaning on primary nutrient concentrations. Neither pellet durability index (42.9% and 43.1% for uncleaned and cleaned, respectively) nor pellet fines percentage (7.35% and 7.6% for uncleaned and cleaned, respectively) differed among corn cleaning method.

The interaction between corn type or diet form impacted ($P < 0.05$) ADG from d 14 to 28 of the experiment, but no other measured parameter ($P > 0.05$;Table 9). Pigs fed uncleaned corn had similar ($P > 0.05$) ADG when fed mash or pelleted diets. However, those fed cleaned corn had greater ($P < 0.05$) ADG when feed was pelleted.

Corn type did not impact ($P > 0.05$) nursery pig growth performance. Overall, pellet mill type B had a marginally significant ($P = 0.081$) improvement in ADG compared to the smaller sized pellet mill Type A, however, there were no differences in any other time period or response criteria. When comparing mash diets to all pelleted diets, pigs fed mash diets had greater ($P < 0.05$) ADG from d 0 to 14 than those fed pelleted diets, which was driven by greater ($P < 0.05$) feed intake, but not ($P > 0.05$) G:F. The effect was reversed in the second period, where pigs fed pelleted diets had greater

Table 7. Effects of screen size on fumonisin concentration[1]

	Screen size (mm)					
Item	Uncleaned corn	12.7[2]	4.8[3]	12.7 + 4.8[4]	SEM	P
LC–MS/MS analysis						
n	10	3	3	10	–	–
Aflatoxin (total), ppb	<20	<20	<20	<20	–	–
B_1, ppb	<20	<20	<20	<20	–	–
B_2, ppb	<20	<20	<20	<20	–	–
G_1, ppb	<20	<20	<20	<20	–	–
G_2, ppb	<20	<20	<20	<20	–	–
Deoxynivalenol (DON), ppb	<200	<200	<200	<200	–	–
Fumonisin (total), ppm	5.48[a]	5.17[a]	3.74[b]	3.33[b]	0.32	<0.0001
B_1, ppm	4.51[a]	4.27[a]	3.08[b]	2.72[b]	0.38	<0.0001
B_2, ppm	0.97[a]	0.91[a]	0.66[b]	0.60[b]	0.05	<0.0001
Trichothecene (HT-2), ppb	<200	<200	<200	<200	–	–
Trichothecene (T-2), ppb	<20	<20	<20	<20	–	–
Ochratoxin A, ppb	<20	<20	<20	<20	–	–
Sterigmatocystin, ppb	<20	<20	<20	<20	–	–
Zearalenone, ppb	<50	<50	<50	<50	–	–
Rapid technique analysis						
n	20	10	10	10	–	–
Fumonisin (total), ppm	3.73[a]	3.76[a]	2.52[b]	2.58[b]	0.20	<0.0001

[1]A single load of naturally contaminated corn was cleaned by mechanical sieving. Corn was divided into twenty 150 kg runs. Run were randomly assigned experimental treatments and the corn cleaner was sanitized between run. Three 5 kg corn samples were collected from each run, ground, split, and analyzed for mycotoxin concentration by either LC–MS/MS or rapid technique analysis.

[2]Values represent material passing through the 12.7 mm screen.

[3]Values represent material not passing through the 4.8 mm screen.

[4]Values represent material not passing through the 4.8 mm screen, but passing through the 12.7 mm screen.

[a-c]Means within a row with different superscripts differ $P<0.05$.

($P < 0.05$) ADG, lower feed intake, and improved G:F compared to pigs fed mash diets. Overall, pigs fed pelleted diets utilized less ($P < 0.05$) feed, but grew more efficiently ($P < 0.05$), and therefore had similar ($P > 0.05$) ADG as those fed mash diets.

DISCUSSION

Overall Cleaning Implications

In our study, the concentration of aflatoxin and fumonisin were reduced by 26% and 42%, respectively, by cleaning corn using perforated screens. These results are similar to Scudamore and Patel (2000), who found a 40% and 32% reduction in aflatoxin and fumonisin, respectively, when 140 samples of corn received at ports or mills were surveyed before and after cleaning. The aflatoxin and fumonisin that remains in the corn after cleaning is likely present in the whole kernel. This is supported by research reported by Brekke *et al.* (1975), Broggi *et al.* (2002), and Saunders *et al.* (2001). In these studies, the bran and germ contained 2.1 times greater aflatoxin and 2.7 times greater fumonisin than the whole kernel, while the flour contained the least amount of mycotoxin. The bran and germ that are removed from the flour milling process are often used in livestock diets. Including these corn milling by-products in livestock diets holds risk to increase the overall mycotoxin concentration within the diet. Additionally, some toxins in livestock feed fractions may have the potential to become residues in animal products (i.e. aflatoxins, ochratoxin A) and still enter the human food chain (Bullerman *et al.*, 2007). Our study differed from those previously published as the bran and germ were still intact to the kernel, which is common for grain elevator storage because removing these fractions reduces nutritive and economic value.

Alternative cleaning methods are needed to reduce the maximum amount of mycotoxin without reducing nutritive value. Shetty and Bhat (1999) showed when natural fumonisin contaminated corn was immersed in water for 5 minutes, fumonisin B_1 concentration were reduced by 74%. When the same source of corn was immersed in a 30% NaCl solution, fumonisin B_1 concentration were reduced by 86%. Additionally, studies by Bullerman *et al.* (2007), and Pearson *et al.* (2004) demonstrated that high-speed dual-wavelength sorting on high

Table 8. Effects of fraction type on fumonisin concentration[1]

	Fraction type				
Item	Overs[2]	Cleaned corn[3]	Thrus[4]	SEM	P
Percentage of weight, %	0.02	97.76	2.22	–	–
LC–MS/MS analysis					
n	1	10	3	–	–
Aflatoxin (total), ppb	<20	<20	<20	–	–
B_1, ppb	<20	<20	<20	–	–
B_2, ppb	<20	<20	<20	–	–
G_1, ppb	<20	<20	<20	–	–
G_2, ppb	<20	<20	<20	–	–
Deoxynivalenol (DON), ppb	<200	<200	<200	–	–
Fumonisin (total), ppm	21.0[b]	3.33[c]	65.4[a]	1.89	<0.0001
B_1, ppm	18.4[b]	2.72[c]	52.4[a]	1.54	<0.0001
B_2, ppm	2.59[b]	0.60[c]	13.0[a]	0.42	<0.0001
Trichothecene (HT-2), ppb	<200	<200	<200	–	–
Trichothecene (T-2), ppb	<20	<20	<20	–	–
Ochratoxin A, ppb	<20	<20	<20	–	–
Sterigmatocystin, ppb	<20	<20	<20	–	–
Zearalenone, ppb	<50	<50	<50	–	–
Rapid technique analysis					
n	0	10	10	–	–
Fumonisin (total), ppm	–	2.58[b]	70.8[a]	0.90	<0.0001

[1]A 5 kg sample were collected from each run, ground and analyzed for mycotoxin concentration.

[2]Product that did not pass through a 12.7 mm screen.

[3]Product that passed through a 12.7 mm screen but not a 4.8 mm screen.

[4]Product that passed through a 4.8 mm screen.

[a-c]Means within a row with different superscripts differ $P<0.05$.

mycotoxin kernels could reduce aflatoxin and fumonisin by 81% and 85%, respectively. One negative aspect is the high amount (5–10%) of kernels removed during sorting. Despite 5–10% corn removed by sorting, the economic loss may be overcome by developing a safe feed source, especially when handling corn of high mycotoxin concentration.

In addition to aflatoxin and fumonisin, the uncleaned corn also had detectable levels of ochratoxin A. Ochratoxin A concentration was not impacted by cleaning, despite having 2.7 times greater ochratoxin A concentration in the thrus. These findings are contrary to Duarte *et al.* (2010), and Scudamore *et al.* (2003), who reported dust, broken grain, and bran removal lowers the ochratoxin A concentration. In wheat, the ochratoxin A concentration were reduced by 44% after removing screenings and the bran. Our study did not find a difference in ochratoxin A concentration, perhaps because our cleaning methods kept the bran intact when analyzing corn mycotoxin reduction.

In Experiment 1, even after cleaning, aflatoxin content was substantially greater than the maximum tolerance (>300 ppb) allowed for corn to be fed to animals (United States Food and Drug Administration, 1994). With an initial aflatoxin concentration greater than 1,000 ppb, mycotoxin concentration can be difficult to reduce to safe concentrations when using traditional grain handling facility methods. However, the reduction in aflatoxin concentrations observed in this experiment suggests that cleaning corn may be effective when initial concentrations are closer to the maximum tolerance level.

Cleaning corn across a 4.8 mm perforated screen effectively reduced fumonisin concentration, even after initially cleaning with a 12.7 mm screen. Using a 12.7 mm screen was ineffective at reducing mycotoxin concentration. The 12.7 mm screen removed overs while the 4.8 mm screen removed thrus. Since the thrus fraction contained the highest concentration, removing this fraction reduced the overall fumonisin concentration. Even though the overs contained more fumonisin than cleaned corn, the percentage of overs in the entire corn source were less than 0.1%. Pieces of cob in between 4.8 and 12.7 mm in size were found within the cleaned corn sample. Improvements in technology to clean corn not only by size, but by shape and density could prove to further reduce the overall fumonisin

Table 9. Pairwise effects of corn type (unclean or clean) and feed form (mash, type A or type B pellet mill) on growth performance of nursery pigs[1]

Treatment	Mash – Unclean	Mash – Clean	Type A[2] – Unclean	Type A[2] – Clean	Type B[3] – Unclean	Type B[3] – Clean	SEM	P Treatment	P Unclean vs. Clean[4]	P Type A vs. Type B[4]	P Pellet vs. Mash[4]
BW, kg											
d 0	8.8	8.8	8.8	8.8	8.8	8.8	0.130	0.999	0.989	0.951	0.903
d 14	14.6	14.7	14.0	14.1	14.2	14.1	0.272	0.434	0.896	0.811	0.036
d 28	24.1	23.9	23.7	24.0	24.2	24.3	0.366	0.912	0.811	0.337	0.824
d 0 to 14											
ADG, kg	408	420	371	367	388	378	16.9	0.181	0.944	0.392	0.012
ADFI, kg	578[ab]	584[a]	506[c]	518[c]	533[bc]	526[c]	17.2	0.007	0.798	0.308	0.0001
G:F	0.71	0.72	0.73	0.70	0.73	0.72	0.02	0.763	0.452	0.772	0.513
d 14 to 28											
ADG, kg	684[bc]	657[c]	691[bc]	711[ab]	711[ab]	726[a]	12.3	0.003	0.777	0.150	0.001
ADFI, kg	1,074[a]	1,040[ab]	984[c]	1,009[bc]	1,008[bc]	1,014[bc]	18.4	0.020	0.947	0.452	0.001
G:F	0.64[b]	0.63[b]	0.70[a]	0.71[a]	0.71[a]	0.72[a]	0.007	<0.0001	0.654	0.324	<0.0001
d 0 to 28											
ADG, kg	546	538	529	532	550	552	11.6	0.637	0.939	0.081	0.905
ADFI, kg	826[a]	812[ab]	743[c]	758[c]	770[bc]	770[bc]	15.9	0.003	0.998	0.218	<0.0001
G:F	0.66[b]	0.66[b]	0.71[a]	0.70[a]	0.71[a]	0.72[a]	0.007	<0.0001	0.681	0.169	<0.0001

[1] A total of 360 pigs (average initial BW = 8.8 kg) were used in a nursery trial with 5 pigs per pen and 12 replicates per treatment.

[2] Master model 1000 HD (California Pellet Mill Co., Crawfordsville, IN)

[3] Model 3016-4 (California Pellet Mill Co., Crawfordsville, IN).

[4] Each contrast compared the following treatments: 1) 'Uncleaned vs. Cleaned' compared the three unclean treatments to the three clean treatments; 2) 'Type A vs. Type B' compared the 2 type A treatments to the 2 type B treatments; 3) 'Pellet vs. Mash' compared the two mash treatments to the four pelleted treatments.

[abcd]Means within a row with different superscripts differ $P < 0.05$.

content since the overs are more concentrated than the cleaned corn.

Screenings

The mycotoxin concentration of the overs fraction was variable across mycotoxin type. Compared to uncleaned corn, overs contained 3.6 times less total aflatoxin, 1.9 times greater total fumonisin, and identical concentrations of Ochratoxin A. Although overs contained 1.9 times greater total fumonisin than uncleaned corn, this was not a large enough impact to reduce the average total fumonisin concentration. In this experiment, based on the concentration of fumonisin within overs, it may be possible to see a reduction in total fumonisin concentration from sources of corn that contain a higher percentage of overs. While trichothecene and sterigmatocystin were undetected in the uncleaned corn, there were detectable levels within overs. Trichothecene and fumonisin are generally produced in growing cereal crops, while sterigmatocystin and ochratoxin A are produced during storage. When grains are received, screening may inhibit the accumulation of sterigmatocystin and ochratoxin A during storage.

In experiment 1, thrus had 7.5 times greater fumonisin concentration than uncleaned corn, while in experiment 2 the thrus contained 19.6 times more fumonisin, compared to uncleaned corn. These findings are supported by Murphy *et al.* (1993), who analyzed 160 total samples of corn from multiple states. They found that thrus contained approximately 10 times higher fumonisin B_1 concentration than uncleaned corn. The determination that thrus contain the highest mycotoxin concentration appears to be consistent, although their mycotoxin concentration can be variable.

The mycotoxin concentration in the screenings are 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. If storing whole corn contaminated with aflatoxins or fumonisins, 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. When cleaning grains, shrink can

be affected because small and large particles are removed. For this reason, not all manufacturers separate screenings. It is common practice to feed screenings, mostly utilized by ruminants, either as a distinct commodity or by addition to the ground corn bin, which can lead to high risk pulses of mycotoxicosis. For these reasons, caution should be taken with the screenings removed during cleaning.

Swine Growth Performance

While physically cleaning corn is shown in this project to reduce mycotoxin content, it is unclear if this change has the potential to impact the growth performance of swine. The current experiment suggests that cleaning corn does not affect the nutrient density or pellet quality of swine diets. This is likely due, in part, to the small proportion of overs and thrus in uncleaned corn. These results suggest that removing screenings from corn naturally contaminated with fumonisin does not affect average swine ADG or feed efficiency. This is likely because initial fumonisin concentrations were far below the 20 ppm threshold that has been shown to negatively affect growth performance (Rao *et al.*, 2020). Therefore, these data suggest that screenings from low mycotixin loads of corn can be included in swine rations without affecting growth performance.

Pelleting improved overall feed efficiency. This improvement in feed efficiency was driven by reduced ADFI in pigs fed pelleted diets, while there was no difference in ADG across feed form. These results support Jensen *et al.* (1965), who found that pigs fed a pelleted corn-soybean meal-based diet had improved G:F, but no differences in ADG, which was driven by reduced ADFI. Interestingly in our study from d 0 to 14, pigs fed pelleted diets conditioned at 85˚C had lower ADG compared to mash diets, but from d 14 to 28 pelleted diets had higher ADG. Overall pigs fed pelleted diets had lower ADFI compared to pigs fed mash diets. This is expected because G:F increased. This improvement in G:F could be partially due to improved dry matter, nitrogen, gross energy digestibility, and apparent ileal digestibility (AID) of starch and most AA (Wondra, 1995; Rojas *et al.*, 2015).

In the current experiment, pellet mill type did not influence overall feed efficiency. De Jong et al. (2014) reported conflicting results with regard to the effect of pellet mill type on nursery pig growth performance. In one experiment, ADG and feed conversion were impacted by pellet mill, but this effect was not observed in a separate experiment. These results are inconsistent, and the operator, conditioning temperature, conditioner retention time, and die specification may influence nursery pig growth performance if these parameters are not held constant when comparing pellet mill types. In our experiment, operator, pellet processing parameters, and facility were kept the same for all pelleted diets.

In conclusion, screening corn is one method to reduce aflatoxin and fumonisin concentration without impacting dietary nutrient density or pellet quality. The average aflatoxin and fumonisin concentration of corn containing 5.86% of the thrus fraction can be reduced by approximately 36% and 45%, respectively, when performing a single pass across a single 4.8 mm screen. Additionally, in corn that contains 2.2% of the thrus fraction, the same cleaning method can effectively reduce fumonisin concentration by 32%. In Experiments 1 and 2, the screenings contained the highest mycotoxin concentration. Yet, neither cleaning low fumonisin concentrated corn nor pellet mill type affected nursery pig growth performance. In years of high mycotoxin grain, screenings should be discarded or utilized for an alternative processing method that does not create livestock feed. While it is common for the feed industry to feed screenings to livestock, these results suggest that feeding screenings may lead to a heightened risk for mycotoxicosis.

Conflict of interest statement. None declared.

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