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## ZEARALENONE: DISTRIBUTION IN DRY-MILLED FRACTIONS OF CONTAMINATED CORN[1]

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Three lots of naturally contaminated yellow corn from the 1972 crop were dry milled to determine the distribution of zearalenone in mill fractions. Two different procedures were used to mill representative samples of the three lots of contaminated corn. Zearalenone and fat contents of each fraction were determined, and their distributions calculated. Dry cleaning of the corn before milling removed from 3 to 10% of the zearalenone. All mill fractions from both procedures were contaminated with zearalenone. The highest levels of contamination were in the hull and high-fat fractions. Prime product mix (grits, low-fat meal, and flour, representing product yields of 57–63%) contained approximately 20% of the zearalenone in the whole corn.

Zearalenone [6-(10-hydroxy-6-oxo-*trans*-1-undecenyl) β-resorcyclic acid lactone] is an estrogenic metabolite produced by a number of *Fusarium* species colonizing corn and other cereal grains. Stob *et al.* (1) demonstrated that this anabolic uterotrophic compound was produced by *Gibberella zeae,* the perfect stage of *Fusarium graminearum.* Urry *et al.* (2) were the first to refer to this material as zearalenone; it is also known as F-2 toxin (3). The estrogenic effects on swine and rats have been documented by Christensen *et al.* (3) and Mirocha *et al.* (4,5,6). Estrogenic disturbances in cattle and swine, which usually occur in the spring and summer, have been associated with the feeding of moldy corn from the previous year's crop (3,7). Mirocha and Christensen (8) have shown that zearalenone is physiologically significant at 500 ppb, based on estrogenic response in swine. Also, sows and gilts receiving 5 mg purified zearalenone daily throughout the last month of pregnancy produced litters with stillborn pigs, or pigs with a "splayleg" incoordination of the hind limbs (9). These same experimental animals, however, produced normal litters when they were maintained on a toxin-free feed.

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[2]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

A 1973 survey by the Food and Drug Administration for *Fusarium* toxins in the 1972 corn crop reported a 17% incidence of zearalenone contamination (100–5,000 ppb) in the northern corn belt (10). As a result of these findings, we studied the distribution of zearalenone in mill fractions from contaminated corn dry milled by two procedures: Northern Regional Research Laboratory (NRRL), and John Stuart Research Laboratories (JSRL), Quaker Oats Company.

## MATERIALS AND METHODS

### Sample Collection

Eleven lots (500–600 lb) of 1972 yellow dent corn from central Illinois were examined in the summer of 1973. These lots were collected as the corn was delivered to the elevator from individual farms. From each lot, 10-lb samples were taken for preliminary zearalenone assay. On the basis of these assays, three lots of corn containing different levels of zearalenone were selected for dry-milling studies.

### Preliminary Procedures

The three lots of corn were blended individually and 50-lb samples removed from each lot. Portions (5 lb) of these samples were taken for grade determination by USDA standard procedures (11), and the remainder (45 lb) was ground to −20 mesh. Ground samples were reblended and 50-g subsamples taken for zearalenone assay. Values were recorded as the levels of zearalenone contamination in the corn lots, as received and blended.

Each lot of corn was cleaned in the air-screen cleaner (*i.e.*, a fanning mill or simple milling separator) (12), and the coarse and fine screenings (1–6 lb) saved for zearalenone assay. Each cleaned corn lot was reblended and a 50-lb sample removed. A second 5-lb portion (cleaned, blended corn) was taken for grade determination. The remainder was ground to −20 mesh, reblended, and a subsample (50-g) taken for zearalenone assay. These values were recorded as zearalenone levels in the corn lots as milled.

Microbiological evaluation was carried out on surface-sterilized kernels from each lot of corn (as received and blended). Fifty kernels were plated on potato dextrose agar (PDA) and incubated at 28°C for 5 days. Percent infection by *Gibberella zeae* and total Fusaria were determined for each lot of corn.

### Dry-Milling Procedures

*NRRL.* Clean, blended corn (35 lb) was tempered at room temperature to 21% moisture for 1-3/4 hr and then to 24% moisture for 1/4 hr. The tempered corn was processed through an experimental horizontal drum degermer (12), fitted with a 20/64-in. round-hole perforated (rhp) screen and a rotor operating at 1750 rpm. Throughput was 4–5 bu/hr, tempered corn basis. The degermer stock was dried to 16–18% moisture in a forced-air dryer at 100°F. Approximately 23 lb of dried degermer stock was roller milled as described by Brekke *et al.* (12), with the following exceptions: 1) a Sweco vibratory sifter was substituted for the box sifter except for removal of +3-1/2 recycle fraction and at the tailings stage, 2) a 20-mesh sieve was substituted for the 25-mesh sieve at each stage, 3) a 50-mesh sieve was added at the second break and first germ operations

to separate low-fat meal and low-fat flour fractions, and 4) a 16-mesh sieve instead of a 14-mesh sieve was used at the third break for the first germ stream.

Product streams were dried to 10–12% moisture in a forced-air dryer (140° F). Mill stream weights were determined and yields calculated. Mill streams were blended and 1-kg quantities (when available) taken for analyses. The unused dried degermer stock (*ca.* 8 lb) was ground, blended, and sampled for zearalenone analysis. The milling equipment was thoroughly cleaned with 1% sodium hypochlorite or 70% ethanol to prevent contamination from other runs.

*JSRL.* Cleaned, blended samples (*ca.* 1500 g each) from the three lots of corn were tempered to 14–15% moisture for 24 hr or longer. The tempered corn samples were milled by the experimental flow mill shown in Fig. 1. The degermer output was sieved for 2 min on the AC sifter and fraction weights were recorded. The +5 wire fraction was rerun through the degermer at 3000 rpm and sieved; weights were recorded. Fractions were combined (see flowsheet) and aspirated in a Bates aspirator.

The +5 and +7 wire fractions were run through the water column to separate grits and germ. Fractions separated by the water column were dried down for further milling steps. Weights were determined for total grits, meal, flour, germ, and feed fractions. Fractions were individually blended and analyzed for zearalenone and fat content.

**Analytical Procedures**

Appropriate samples of cleaned, blended whole corn were taken for proximate analyses: fiber, starch, ash, and crude fat (13); protein (14); soluble solids (15); and fat acidity (16).

Fat content of all mill fractions except germ were determined by the chromatographic method of Black *et al.* (17). Germ fat was determined according to AACC Methods (13).

Zearalenone assays were done according to Eppley's procedure (18) with the following modification: The entire extract was collected by vacuum filtration and the filtrant washed with three 250-ml volumes of chloroform. Combined filtrate and washes were reduced to less than 250 ml on a flask evaporator (37° C) and then diluted to 250 ml with chloroform; 50 ml was taken for column chromatographic isolation of zearalenone (18). Zearalenone so isolated was assayed by thin-layer chromatography (tlc) on plates coated with Silica Gel GHR (0.5 mm). Sample extracts in 0.5 ml benzene were spotted at three levels (usually 2, 5, and 10 $\mu$l) adjacent to three levels (3, 5, and 7 $\mu$l) of standard zearalenone (100 $\mu$g/ml benzene). An admixture of each sample and the standard was also spotted. Plates were developed in ethanol:chloroform (5:95 v/v), air dried, and examined under shortwave ultraviolet light (253.7 nm). The quantity of zearalenone in the sample was estimated by visual comparison of sample zones to matching standard zones. When samples contained high levels of zearalenone, appropriate dilutions were made and the tlc procedure was repeated. This method is routinely sensitive to 100 $\mu$g/kg. Reported values are averages of duplicate (or more) determinations.

## RESULTS AND DISCUSSION

Corn lots chosen for this study were selected from an area in which the Food

and Drug Administration (FDA) had previously found zearalenone-contaminated corn (10). The 1972 corn crop in this area was subjected to unusually wet conditions before and during harvest, conditions which favor fungal contamination particularly by Fusaria. Grade evaluation, zearalenone content, and *Fusarium* contamination in the corn lots are given in Table I. Other molds observed were: *Aspergillus flavus* and species of *Nigrospora*, *Penicillium*, and *Cladosporium*. As the percentage of *Fusarium* infection increased, the level of zearalenone increased and corn quality decreased. Lots 28-2 and 14-2B were graded No. 2 and 4, respectively, both before and after cleaning. Lot 18-1 dropped from No. 5 to Sample Grade owing to excessive amounts of damaged kernels (16% DKT). The test weight on each lot was approximately 58 lb at 13–15% moisture. Broken corn and foreign material (BCFM) ranged between 0.3 and 0.5% in the corn as received and was 0.2% or less in cleaned corn. Physical

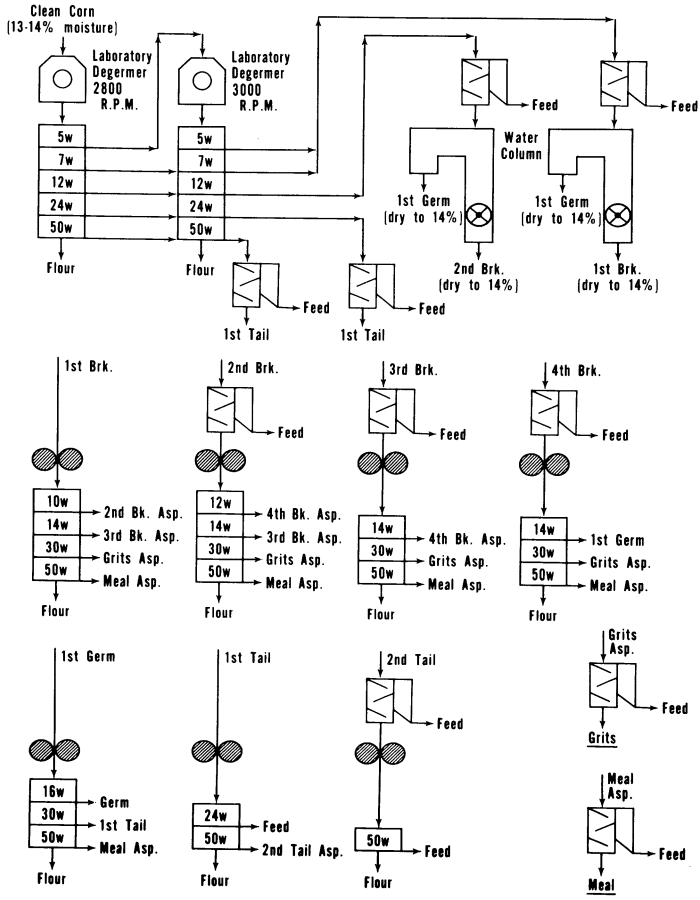


Fig. 1. Flow sheet for dry milling zearalenone-contaminated corn. w = wire mesh; Bk = break; Asp = aspirator. Source: John Stuart Research Laboratories, The Quaker Oats Company.

appearance of lot 18-1 indicated considerable mold damage, and such a sample would not be typical of corn used for dry milling. Lots 28-2 and 14-2B appeared to be typical yellow dent corn with some damaged kernels.

The degree of *Fusarium* damage generally correlates with zearalenone levels. On the basis of mold damage and discoloration, handpicked kernels from the three lots of corn illustrate this correlation. Kernels with the least visible germ damage contained low levels (100 to 450 ppb) of zearalenone. Purple and brown kernels with heavy mold damage contained zearalenone levels of 23,000 to 57,200 ppb. The high levels of zearalenone in a few kernels require that large representative samples of a contaminated lot be analyzed to determine actual zearalenone levels.

**TABLE I**
**Grade, Zearalenone Level, and Fusarium Infection of the Three Lots of Corn Used in Dry-Milling Studies**

| Lot No. | U.S.D.A. Grade No. Before/After Cleaning | Zearalenone ppb[a] | *Gibberella zeae* Infection % | All Fusaria Infection % |
|---|---|---|---|---|
| 28-2 | 2/2 | 925 | 0 | 22 |
| 14-2B | 4/4 | 3500 | 12 | 36 |
| 18-1 | 5/SG[b] | 7800 | 16 | 52 |

[a]Zearalenone content of corn as received and blended; ppb.
[b]SG = Sample Grade.

**TABLE II**
**Yields, Fat Contents, Zearalenone Levels, and Zearalenone Distribution in Cleanings from Contaminated Corn**

| Fraction | Lot No. 28-2 | 14-2B | 18-1 |
|---|---|---|---|
| Cleaning yields, % | | | |
| Corn, dry cleaned | 99.0 | 98.4 | 99.1 |
| Oversize screenings | 0.3 | 0.4 | 0.2 |
| Undersize screenings | 0.7 | 1.2 | 0.7 |
| Fat contents, % db | | | |
| Corn, dry cleaned | 4.3 | 3.9 | 4.1 |
| Oversize screenings | 1.6 | 1.7 | 1.1 |
| Undersize screenings | 2.2 | 3.3 | 3.2 |
| Zearalenone levels, ppb | | | |
| Corn, as received | 925 | 3,500 | 7,800 |
| Corn, dry cleaned | 775 | 3,500 | 8,100 |
| Oversize screenings | 15,500 | 9,300 | 34,900 |
| Undersize screenings | 6,200 | 9,300 | 27,200 |
| Zearalenone distribution, %[a] | | | |
| Corn, dry cleaned | 90 | 96 | 97 |
| Oversize screenings | 5 | 1 | 1 |
| Undersize screenings | 5 | 3 | 2 |

[a]Based on zearalenone level in dry cleaned corn.

Cleaning the three lots of corn removed 0.9–1.6% of the grain as screening fractions but did not significantly reduce the zearalenone content in the corn (Table II). A 26/64-in. rhp screen removed oversize screenings and a 15/64-in. rhp screen removed undersize screenings. Oversized screenings contained zearalenone levels from 9,300 to 34,900 ppb. Undersized screenings, produced in quantities two to three times that of the oversized screenings, contained levels from 6,200 to 27,200 ppb. Based on contamination levels in the cleaned corn, the distribution of zearalenone in the screenings varied from 3% (lot 18-1) to 10% (lot 28-2). Fat content of the screenings did not correlate with zearalenone levels.

Proximate chemical analyses of whole corn samples from the three lots are given in Table III. The fat, ash, fiber, and soluble solids are similar in the three lots and are typical of yellow dent corn. The fat acidity values are normal for lots 28-2 and 14-2B; however, the value for 18-1 is high (109) and reflects oil deterioration in the germ. This increase in acidity is probably caused by mold lipase activity.

Table IV lists the product yields, zearalenone, fat, and zearalenone distribution among the mill fractions produced by the NRRL procedure. Calculations were based on total quantities of milled fractions and zearalenone recovered. Roller milling response by the NRRL procedure was similar to that of yellow dent corn contaminated with aflatoxin (19). Grit yields ranged from 35 to 39% and prime product yields (Mix No. 1) were 58–60%. Fractions that are generally incorporated into hominy feed (hull, bran meal, degermer fines) accounted for 12–14% of total product. The germ fraction varied from 14 to 17% of total product.

Grit fat content was comparable (0.7% or less for each lot) to the levels in grits from good quality corn. Germ fat content ranged from 20 to 24% and calculated yields of recoverable oil varied from 2.2 to 2.6 lb/net cwt of corn milled.

Zearalenone levels in milled fractions increased as the levels in the corn increased. Grit fractions always contained the lowest levels of zearalenone, and these levels were 1/4–1/8 the concentration in the corn. Prime product mix (Mix No. 1) accounted for 58–60% of yield of milled product, but contained only 10% (lot 28-2) to 22% (lot 18-1) of total zearalenone. Germ contained the highest levels of zearalenone found in the milled fractions (2,400 ppb in lot 28-2 to 18,400 ppb in lot 18-1). These levels were two to three times the levels in the whole corn. The germ and hominy feed fractions contained 60–70% of the zearalenone contamination.

**TABLE III**
**Proximate Analyses of Whole Corn Samples[a]**

| Constituent | Lot No. | | |
|---|---|---|---|
| | 28-2 | 14-2B | 18-1 |
| Protein | 9.4 | 7.3 | 10.3 |
| Fat | 4.3 | 3.9 | 4.1 |
| Ash | 1.5 | 1.3 | 1.4 |
| Fiber | 2.5 | 2.3 | 2.2 |
| Starch | 70.8 | 74.5 | 70.0 |
| Soluble solids | 5.8 | 5.3 | 4.9 |
| Fat acidity | 31[b] | 59[b] | 109[b] |

[a]All assays reported as % dry basis; cleaned, blended corn.
[b]mg KOH/100 g dry matter in corn.

Considering the variability due to sampling, subsampling, and analytical procedures, there was good agreement between calculated zearalenone content of composited milled fractions and corn as milled. Agreement between these values and those of the degermer stock was good except for lot 14-2B. Analysis of a second stock sample gave low results, indicating the source of error to be in the initial sampling of this lot for milling.

Different product yields and fat content of fractions were obtained by the JSRL procedure (Table V). When compared to NRRL, the JSRL procedure yielded more degermer flour, no high-fat meal and flour, less germ, and a feed fraction exceeding the combined NRRL yields of hull and bran meal. However, when mill fractions were grouped as mixes, there was considerable agreement in the data for yields, fat content, and zearalenone distribution.

## SUMMARY AND CONCLUSIONS

Three lots of zearalenone-contaminated corn (USDA grade No. 2, 4, and

**TABLE IV**
**Product Yield, Fat Content, Zearalenone Level and Distribution in Fractions from Corn Dry Milled by NRRL[a]**

| | Yields, % np[b] | | | Fat, % db | | | Zearalenone ppb | | | Zearalenone, % of Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Product** | **28-2** | **14-2B** | **18-1** | **28-2** | **14-2B** | **18-1** | **28-2** | **14-2B** | **18-1** | **28-2** | **14-2B** | **18-1** |
| Grits | 37 | 35 | 39 | 0.7 | 0.6 | 0.7 | 100 | 625 | 2,100 | 5 | 8 | 13 |
| Low-fat meal | 18 | 19 | 19 | 0.7 | 0.6 | 1.1 | 150 | 775 | 2,400 | 3 | 5 | 7 |
| Low-fat flour | 3 | 5 | 2 | 1.1 | 0.5 | 1.3 | 350 | 925 | 4,600 | 2 | 2 | 2 |
| Mix No. 1 | 58 | 59 | 60 | 0.7[c] | 0.6[c] | 0.9[c] | 125[c] | 700[c] | 2,300[c] | 10 | 15 | 22 |
| High-fat meal | 10 | 10 | 10 | 1.8 | 1.2 | 1.9 | 1,200 | 6,200 | 7,900 | 16 | 22 | 13 |
| High-fat flour | 3 | 2 | 3 | 1.6 | 0.7 | 1.8 | 1,200 | 3,500 | 9,200 | 4 | 3 | 4 |
| Mix No. 2 | 13 | 12 | 13 | 1.7[c] | 1.1[c] | 1.9[c] | 1,200[c] | 5,700[c] | 8,200[c] | 20 | 25 | 17 |
| Mix No. 1 + 2 | 71 | 71 | 73 | 0.9[c] | 0.7[c] | 1.1[c] | 300[c] | 1,600[c] | 3,400[c] | 30 | 40 | 39 |
| Hull | 6 | 8 | 7 | 1.2 | 1.7 | 1.5 | 1,200 | 5,600 | 7,900 | 10 | 16 | 9 |
| Bran meal | 4 | 3 | 4 | 2.7 | 3.0 | 3.2 | 600 | 4,600 | 8,500 | 3 | 5 | 5 |
| Germ | 17 | 14 | 15 | 20.6 | 23.7 | 20.4 | 2,400 | 7,000 | 18,400 | 53 | 34 | 44 |
| Degermer fines | 2 | 3 | 1 | 1.7 | 0.8 | 2.1 | 1,200 | 5,600 | 14,000 | 3 | 5 | 2 |
| Composite calc'd | | | | | | | 800[c] | 2,900[c] | 6,300[c] | | | |
| Degermer stock | | | | | | | 650 | 1,700 | 7,900 | | | |
| Corn, dry cleaned | | | | | | | 800 | 3,500 | 8,100 | | | |

[a]Data obtained by NRRL—Northern Regional Research Laboratory.
[b]np—Net product (gross product less recycle fraction).
[c]Weighted average.

TABLE V
Distribution of Products, Zearalenone, and Fat Content in Corn Dry Milled by JSRL[a]

| Fraction | Yields, % np | | | Fat, % wb[b] | | | Zearalenone ppb | | | Zearalenone, % of Total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 28-2 | 14-2B | 18-1 | 28-2 | 14-2B | 18-1 | 28-2 | 14-2B | 18-1 | 28-2 | 14-2B | 18-1 |
| Corn as milled | | | | | | | 300 | 1,700 | 7,200 | | | |
| Grits | 44 | 39 | 44 | 0.9 | 0.8 | 1.0 | 50 | 50 | 500 | 3 | 1 | 3 |
| High-fat meal | 7 | 6 | 7 | 0.7 | 0.4 | 1.2 | 50 | 250 | 1,300 | 1 | 1 | 1 |
| Mill flour | 11 | 12 | 12 | 2.0 | 1.3 | 2.1 | 200 | 1,100 | 600 | 3 | 8 | 1 |
| Mix No. 1 | 62 | 57 | 63 | 1.0[c] | 0.9[c] | 1.2[c] | 80[c] | 300[c] | 600[c] | 7 | 10 | 5 |
| Degermer flour | 9 | 14 | 8 | 1.4 | 1.3 | 1.5 | 2,100 | 4,400 | 10,800 | 29 | 35 | 12 |
| Mix No. 2 | | | | | | | | | | | | |
| Mix No. 1 + 2 | 71 | 71 | 71 | 1.1[c] | 0.9[c] | 1.3[c] | 300[c] | 1,100[c] | 1,800[c] | 36 | 45 | 17 |
| Germ | 11 | 11 | 10 | 23.8 | 21.8 | 22.1 | 2,200 | 4,700 | 37,500 | 37 | 28 | 47 |
| Feed | 18 | 18 | 20 | 3.8 | 2.9 | 3.5 | 950 | 2,800 | 14,400 | 26 | 28 | 36 |
| Mix No. 3 | 29 | 29 | 30 | 11.3 | 10.1 | 9.7 | 1,400[c] | 3,500[c] | 22,000[c] | 63 | 56 | 83 |
| Composite (calc'd) | 100 | 100 | 101 | 4.0[c] | 3.5[c] | 3.7[c] | 700[c] | 1,800[c] | 7,700[c] | 99 | 101 | 100 |

[a]Data obtained by JSRL—John Stuart Research Laboratories, Quaker Oats Company.
[b]wb—Wet basis.
[c]Weighted average.

Sample Grade, respectively) were dry milled by two procedures. Dry cleaning removed from 3 to 10% of the zearalenone contamination. All product fractions produced by dry milling of cleaned corn were contaminated with zearalenone. Grit fractions contained the lowest levels of contamination, and germ and feed fractions contained the highest levels of contamination.

Data from these milling studies showed considerable variability in results, apparently due to sampling and analytical techniques. Consequently, adequate sampling and analytical procedures should be developed for rapid detection of zearalenone in corn. Although widespread *Fusarium* damage may occur infrequently, methods to determine possible toxin contamination must be available.

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