Natural occurrence of zeralenone in feeds and feedstuffs used in poultry and pig nutrition in Colombia

Gonzalo J. Diaz[1] and Angel E. Céspedes

Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia. Postal address: Apartado Aéreo 76948, Santafé de Bogotá, D.C. Colombia, South America.

[1] To whom correspondence should be addressed

Abstract

A total of 200 samples of feedstuffs and mixed feeds used for poultry and pig nutrition in Colombia were analysed for zearalenone using a LC technique with a limit of detection of 20 µg/kg. Samples of grain sorghum, maize, processed soybean, rice meal, cottonseed meal, and poultry and pig feeds, representative of the Colombian production for the 1995-1996 harvest, were taken at feed manufacturing plants located in different cities of the country. Zearalenone was detected in 25 of 45 samples of sorghum, 2 of 33 samples of maize, 7 of 22 samples of rice meal, 9 of 17 samples of cottonseed meal, 11 of 30 samples of poultry feed and 6 of 16 samples of pig feed. Zearalenone was not detected in soybean or other feedstuffs analysed (wheat by-products, cassava meal, palm). Overall levels of zearalenone ranged from 29 to 3956 µg/kg, with a mean value of 436 µg/kg. Only one of the 6 positive samples of pig feed had a zearalenone concentration above 500 µg/kg, which is normally considered as the safe level for pigs.

Introduction

Zearalenone (ZEA) is a phenolic resorcyclic acid lactone produced by several strains of Fusaria, including *F. graminearum, F. tricinctum, F. oxysporum, F. sporotrichioides and F. moniliforme* (1). Even though ZEA is a non-steroidal metabolite, it has estrogenic-like effects and was determined to be the cause of reproductive problems in swine being fed mouldy corn (2). ZEA is mainly a contaminant in corn, but it may also occur in oats, barley, wheat and sorghum (3). Swine appear to be the domestic animal species most susceptible to natural outbreaks of ZEA toxicosis, being the prepubertal gilts the most sensitive (4). Gross changes induced by ZEA in prepubertal gilts include swelling of the vulva, increased size and weight of the uterus, and mammary enlargement; in extreme cases, rectal and vaginal prolapses occur (4). In sows, ZEA causes reproductive disorders including infertility, constant estrus, pseudopregnancy, diminished fertility, reduced litter size, smaller offspring, malformation, and probably fetal resorption (5).

ZEA appears to be practically non toxic for poultry species (6); however, Branton et al. (7) found a significant decrease in egg production in layers fed a diet containing 1102 µg/kg ZEA and 298 µg/kg deoxinivalenol. On the other hand, the detection of ZEA in poultry feed has been suggested to be used as a "marker" for other yet unknown *Fusarium* toxins (8). The presence of a biomarker, whether toxic or not, indicates that the feed was exposed to conditions favourable for mould growth, which increases the possibility that the feed is contaminated with mycotoxins (8).

The incidence and level of ZEA contamination in various commodities has been monitored worldwide (9). However, to the authors' knowledge, there is no information regarding ZEA contamination in Colombian feeds or feedstuffs. The aim of the present study was to investigate the incidence and level of contamination with ZEA in samples of feeds and feedstuffs used for poultry and pig nutrition in Colombia.

Materials and Methods

Samples

Samples of cereal grains and mixed feeds were taken at feed manufacturing plants located in different cities of the country including Cartagena, Guadalajara de Buga, Medellín, Mosquera, and Santafé de Bogotá. Samples were obtained from five feed companies, which commercialise and produce about 95% of the poultry and pig feeds and feedstuffs in Colombia. Sampling and subsampling of raw materials and mixed feed was conducted according to the recommendations of Campbell et al. (10) and Park & Pohland (11). Samples of raw materials were representative of the Colombian production for the 1995-1996 harvest. Raw materials were sampled as they arrived at the feed manufacturing plant. Samples of mixed feed were taken from stored feed. A total of 200 samples were analysed for ZEA: 45 samples of grain sorghum, 33 samples of maize, 25 samples of soybean, 22 samples of rice meal, 17 samples of cottonseed meal, 12 samples of other feedstuffs, 30 samples of poultry feed and 16 samples of pig feed. The collected samples were stored in paper bags in a cool dry place until they were analysed (within one week of collection).

Analysis

Extraction and cleanup. Samples were extracted by using a slight modification of a previously published procedure (12). A 50 g of ground analytical sample was extracted with 100 ml acetonitrile-H2O (84+16) for 1.0 h using a wrist-action shaker (Multi-Wrist® Shaker, Lab-Line Instruments, Inc., Melrose Park, IL, USA) at high speed (a 25 g sample was taken in the case of hygroscopic feedstuffs such as rice meal). The extract was filtered using qualitative high speed filter paper and 5 ml of the filtered extract were transferred into a 10 ml culture tube. The filtered extract was then acidified with 10 µl glacial acetic acid in order to enhance the recovery of zearalenone; after this, the contents of the tube were gently vortexed for 30 seconds. A multifunctional cleanup column (Mycosep 224 MFC Column, Romer Laboratories, Inc., Union, MO, USA) was used for the purification of the extract. The column was slowly pushed into the culture tube until ca. 1.2 ml of purified extract appeared in the column reservoir, then 1 ml of purified extract was quantitatively transferred to a clean 10 ml culture tube. The purified extract was then evaporated to dryness in a 65°C waterbath under vacuum. The dry residue was dissolved with 1 ml mobile phase (see HPLC analysis), filtered through a type HVLP 0.45 µm pore size hydrophilic membrane (Millipore Corp., Bedford, MA, USA), and 20 µl were injected into LC.

HPLC analysis. The HPLC analysis was based on a previously published method, with some modifications (13). The chromatographic conditions were as follows, mobile phase: isocratic H_3PO_4 0.01M-acetonitrile (57+43) at a flow rate of 0.6 ml/min (Perkin-Elmer

Series 410 LC Pump, Perkin-Elmer Corp., Norwalk, CT, USA); analytical column: reversed phase ODS, 12.5 cm x 4 mm I.D., particle size, 5 µm (LiChrospher® 100 RP-18, Merck KGaA, Darmstadt, Germany); column temperature: 40°C (Perkin-Elmer LC-100 Column Oven, Perkin-Elmer Corp., Norwalk, CT, USA); fluorescence detector: excitation wavelength 270 nm; emission wavelength 465 nm (Perkin-Elmer 650-15 Fluorescence Spectrophotometer, Perkin-Elmer Corp., Norwalk, CT, USA). The limit of detection of the method for ZEA was 20 µg/kg. When blank samples of sorghum, maize, soybean, rice meal, cottonseed meal, poultry feed and pig feed were spiked with 100 or 1000 ng of ZEA per g of each sample, recoveries ranged from 91 to 102%. The quality control included confirmation of 10 positive samples (taken at random) by TLC. Two ml of the purified extract (obtained from the multifunctional cleanup column) were taken to dryness and dissolved with 100 µl of toluene-acetonitrile (95+5), then 20 µl of this solution were spotted on silicagel TLC plates and developed with toluene-acetone (50+50), along with appropriate standards. ZEA spots were visualized under long-wave UV light after dipping the plate in a 15% aluminum chloride solution in methanol (14). Zearalenone standard was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Authenticity of the standard was made by TLC, and quantification by absorption of ZEA in the UV range, using a Shimadzu Model 160 A recording spectrophotometer (Shimadzu Corp., Kyoto, Japan) (15).

Statistical analysis. In order to show graphically the difference in ZEA concentration among the different commodities, Box and Whisker plots were made using a software package (16). It was not possible to make a plot for ZEA levels in maize because only two samples were found positive. Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data (the data between the first and third quartiles) and is bisected by a line at the value of the median. The vertical lines at the top and the bottom of the box are called whiskers, and they indicate the range of typical data values. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers (16). Due to the presence of outliers, a nonparametric two-sample test was used to determine the equality of the median values (median test) (16).

Results and Discussion

Measurable amounts of ZEA were found in sorghum, maize, rice meal, cottonseed meal and mixed feeds. ZEA was not detected in soybean samples or in the "other feedstuffs" (wheat by-products, cassava meal, palm) analysed (Table 1). The overall incidence of ZEA was 30% (60 out of 200 samples), but the incidence was higher in complete feeds (37.0%) than in feedingstuffs (27.9%). Levels of ZEA ranged from 29 to 3956 µg/kg with a mean value of 436 µg/kg. The overall incidence of ZEA found in the present study is lower than the 44% worldwide incidence reported by Tanaka et al. (17), in a survey of 500 samples from 19 different countries. However, the mean value of positive samples found in this latter study (45 µg/kg) was about ten times lower than the corresponding value found in the present survey. Also, ZEA levels found in the survey made by Tanaka et al. (17) ranged from 219 to only 500 µg/kg. The incidence of ZEA in the samples analysed was also much higher than the incidence reported for similar commodities in the United States (US). In surveys conducted for zearalenone in feeds (corn, corn products, wheat, soybean, sorghum) moving in commercial channels in the US, it was found that only 8% of the 1722 samples examined contained measurable amounts of ZEA (3). The incidence of ZEA in Brazil is also lower than the incidence found in the present survey. Baldissera et al. (18) analysed 519 samples of different commodities (maize, rice, sorghum,

Table 1 — Incidence and levels of zearalenone in major feedstuffs and complete feeds used for pig and poultry nutrition in Colombia

Substrate	Samples analysed	Positive samples	Incidence	Median (µg/kg)	Mean (µg/kg)	Range (µg/kg)
Feedstuffs						
Sorghum	45	25	55.6%	385	746	36-3956
Maize	33	2	6.1%	145	145	29-260
Soybean	25	0	0	-	-	-
Rice meal	22	7	31.8%	192	351	44-1621
Cottonseed meal	17	9	52.9%	124	138	57-263
Other feedstuffs[a]	12	0	0	-	-	-
Total feedstuffs	154	43	27.9%	260	526	29-3956
Mixed feeds						
Poultry feed	30	11	36.7%	67	145	44-797
Pig feed	16	6	37.5%	352	323	86-629
Total mixed feeds	46	17	37.0%	86	208	44-797
TOTAL	200	60	30.0%	236	436	29-3956

[a]Wheat by-products, cassava meal, palm meal.

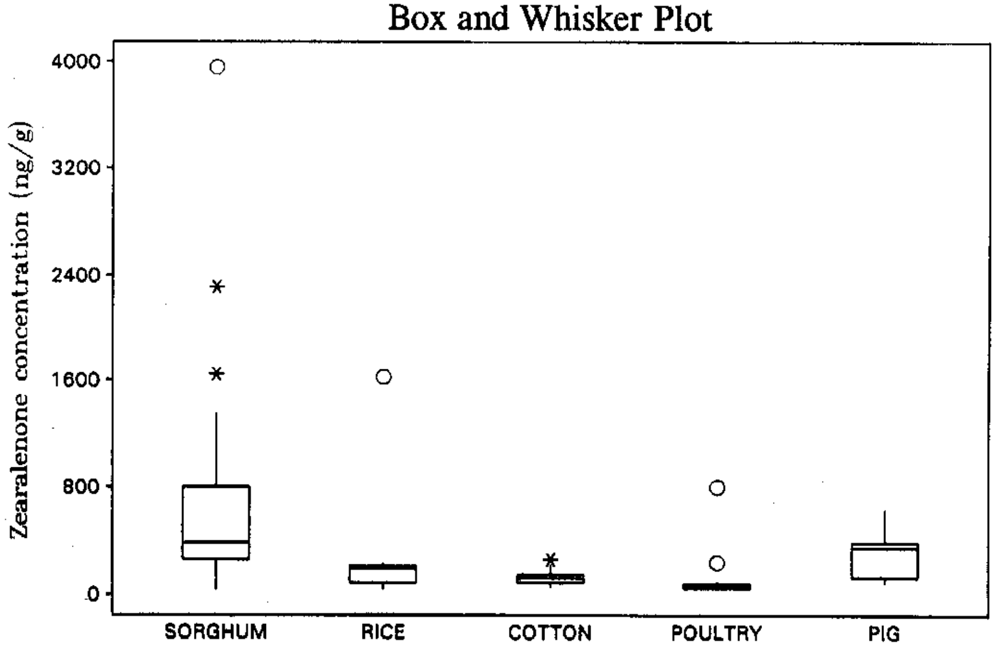

Figure 1 — Zearalenone contamination in feeds and feedstuffs used for poultry and pig nutrition in Colombia (1995/1996).

soybean, wheat and mixed feeds) and found only 25 positive samples (4.8%), with values ranging from 100 to 4982 μg/kg.

Figure 1 shows the Box and Whisker plots for sorghum, rice meal, cottonseed meal, and poultry and pig feeds. Median values among all commodities analysed were significantly different ($P<0.05$).

Feedstuffs

Within the feedstuffs analysed, the highest incidence of ZEA was observed in sorghum grain, where 25 out of the 45 samples (55.6%) contained detectable levels (Table 1). The mean ZEA concentration in positives samples was also the highest for sorghum (746 μg/kg), and the values ranged from 36 to 3956 μg/kg. The incidence of ZEA contamination was higher than the incidence reported for grain sorghum from fields in Georgia and Mississippi (19), where only 30% of 200 samples were contaminated with ZEA; the levels of ZEA in US sorghum were also lower, with levels ranging from 2 to 1468 μg/kg. The second largest incidence of ZEA contamination was found in cottonseed meal (9/17); however, the mean value was the lowest found in positive samples (138 μg/kg), with levels ranging from 57 to 263 μg/kg. About one third of the rice samples were positive for ZEA (31.8%), with values that ranged from 44 to 1621 μg/kg and with a mean of 351 μg/kg. Only two samples of maize resulted positive for ZEA (2/33), which results in an incidence of 6.1%. This finding is consistent with the results of Chulze et al. (20), who found an incidence of ZEA contamination of 6.0% in 150 samples or Argentinean corn, with levels ranging from 40 to 350 μg/kg and a mean value of 210 μg/kg.

Few countries have set tolerances for ZEA, and these vary from 30 to 1000 μg/kg, depending upon the commodity and the country (21). In the present study, only 5 samples of sorghum and one of rice meal exhibited ZEA levels above 1000 μg/kg. The five samples of sorghum with levels above 1000 μg/kg contained 1081, 1343, 1645, 2308, and 3956 μg/kg, respectively. The sample of rice meal had 1621 μg/kg. Obviously, these feedstuffs should be used with caution, particularly when preparing mixed feeds for pigs.

Mixed feeds

The incidence of ZEA contamination in mixed feeds was higher than in feedstuffs (Table 1), which suggest that the mixing of different ingredients in a complete feed increases the likelihood of finding mycotoxins. The overall incidence of ZEA in complete feeds was 37.0% and was very similar for poultry feed (36.7%) and pig feed (37.5%). However, the mean ZEA content in positive samples was lower in poultry than in pig feeds (145 and 323 mg/kg, respectively). Mašic et al. (22) found an incidence of ZEA of 12.03% in 532 pig feed samples analysed over a 5-year period in Yugoslavia. The average contamination level was found to be 368 μg/kg, which is very similar to the mean value observed in the present survey; however, the incidence of ZEA contamination found by Mašic et al. (22) was about one third of the one observed in the present study.

Recommended levels for maximum concentration of ZEA in swine diets are scarce. Diekman et al. (23) recommend that diets for young and growing swine should not exceed 1000 μg/kg. However, Friend et al. (24) reported that prolonged feeding of relatively low levels of ZEA (500 μg/kg) is estrogenic to young gilts and may affect subsequent reproductive efficiency. Mašic et at. (22) indicate that, according to current Yugoslavian regulations, the maximum permitted levels of ZEA are 500 μg/kg in the case of feed intended for piglets and 1000 μg/kg in feed for grown pigs. If it is considered that the no effect level of ZEA in the feed is less than 500 μg/kg, then only one of the positive pig feed samples analysed in the present survey will be regarded as not safe; this sample contained 629 μg/kg.

The significance of finding ZEA levels in poultry diets in not known. However, Romer (8) postulates that ZEA (and deoxinivalenol) can be used as a markers of yet unknown *Fusarium* toxins and suggests that testing for the presence of ZEA may be used to detect and control *Fusarium* field problems in poultry.

Conclusions

The results of the present survey indicate that ZEA is probably an important mycotoxin in Colombian feeds and feedstuffs due to its incidence and levels. The situation is particularly important for sorghum grain because it is widely used in the formulation of pig and poultry rations in Colombia. Permanent monitoring of the ZEA levels in sorghum grain lots produced in Colombia should be advised both for producers and consumers of this grain. Levels of ZEA in rice meal are also of some concern. To keep ZEA contamination at the lower possible levels, surveillance of various feedingstuffs and mixed feeds should continue. In humans, ZEA has been reported as having a possible involvement in cervical cancer and premature telarche (25).Due to the current lack of information, surveys on the incidence of ZEA (and other mycotoxins) in human foods should be conducted in Colombia. Surveys to determine the incidence and levels of mycotoxins other than ZEA and aflatoxin in Colombian foods, feeds and feedstuffs are also needed.

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