Diagnostic notes

Diagnostic assessment and lesion evaluation of chronic deoxynivalenol ingestion in growing swine

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Summary
Deoxynivalenol (DON) is a common mycotoxin contaminant of cereal grains and is associated with reduced feed intake or refusal in swine. The objective of this assessment was to determine if diagnostic tests or lesions could assist in diagnosing chronic DON ingestion in swine. Twenty-four 11-week-old cross-bred pigs of both genders were fed either an ad libitum diet without deliberate contamination of DON (Control; n = 6) or a diet containing approximately 5 mg per kg DON (DON-fed; n = 18). Dried distillers’ grains with solubles were the source of DON for the diets. Serum analytes were measured at the beginning and conclusion of the 120-day study. All pigs were necropsied and liver analyte concentrations, bone density, and bone ash were determined. Differences in serum analyte concentrations, macroscopic or microscopic lesions, and bone ash and density were not detected between treatment groups (P > .05). Liver selenium concentrations were lower (P = .02) in DON-fed pigs. Results suggest DON ingestion is not correlated with lesions or bone integrity, but can significantly lower liver selenium concentrations.

Keywords: swine, deoxynivalenol, DON, selenium, vomitoxin

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Resumen - Valoración diagnóstica y evaluación de lesiones en cerdos de crecimiento de ingestión de deoxynivalenol crónica

El deoxynivalenol (DON por sus siglas en inglés) es un contaminante de micotoxina común de granos de cereal y está asociado con el consumo reducido o rechazo de alimento en cerdos. El objetivo de esta valoración fue determinar si las pruebas de diagnóstico o las lesiones podrían ayudar en el diagnóstico de ingestión crónica de DON en cerdos. Veinticuatro cerdos de 11 semanas de edad, de raza cruzada y de ambos géneros fueron alimentados con dieta ad libitum sin contaminación deliberada de DON (Control; n = 6) o una dieta con contenido de aproximadamente 5 mg por kg de DON (alimentado con-DON; n = 18). La fuente de DON para las dietas fueron granos secos de destiladores con solubles. Se midieron los analitos de suero al principio y a la conclusión del estudio de 120 días. Se hizo necropsia a todos los cerdos y se determinaron las concentraciones de analitos del hígado, y se determinó la densidad y la ceniza de hueso. No se detectaron diferencias en las concentraciones de analitos de suero, lesiones microscópicas o macroscópicas, ni en la densidad y ceniza de hueso entre los grupos de tratamiento (P > .05). Las concentraciones de selenio del hígado fueron más bajas (P = .02) en cerdos alimentados con DON. Los resultados sugieren que la ingestión de DON no está correlacionada con lesiones o integridad del hueso, pero pueden disminuir significativamente las concentraciones de selenio del hígado.

Résumé - Appréciation diagnostique et évaluation des lésions d’ingestion chronique de déoxynivalenol chez des porcs en croissance

Le déoxynivalenol (DON) est une mycotoxine contaminant naturellement les grains de céréales et est associée à une diminution ou un refus de la prise de nourriture chez les porcs. L’objectif de la présente étude était de déterminer si les tests diagnostiques ou les lésions pouvaient aider à diagnostiquer l’ingestion chronique de DON chez le porc. Vingt-quatre porcs croisés des deux sexes et âgés de 11 semaines ont été nourris soit ad libitum avec une alimentation sans contamination intentionnelle par du DON (Témoin; n = 6) ou une alimentation contenant approximativement 5 mg par kg de DON (DON-alimenté; n = 18). Des grains de distillerie partiellement séchés étaient la source de DON pour les rations. Des composantes sériques ont été mesurés au début et à la fin de l’étude de 120 jours. Tous les porcs ont été soumis à une nécropsie et les concentrations de composantes hépatiques, de poudre d’os, et la densité osseuse ont été déterminées. Aucune différence dans les concentrations de composantes sériques, dans les lésions macroscopiques ou microscopiques, la densité osseuse, et de poudre d’os ne fut détectée entre les groupes de traitement (P > .05). Les concentrations hépatiques de sélénium étaient plus faibles (P = .02) chez porcs DON-alimentés. Les résultats suggèrent que l’ingestion de DON n’est pas corrélée avec des lésions ou l’intégrité osseuse, mais peut significativamente faire diminuer les concentrations hépatiques de sélénium.
Doxynivalenol (DON), also known as vomitoxin, is a mycotoxin produced by Fusarium species, which under the correct growing conditions may contaminate cereal grains, especially maize (corn). DON is elevated in cereal grains that are stressed during maturation or harvested with high moisture content, and can increase with inadequate drying and improper storage. The consequence of feeding DON-contaminated feed to livestock can vary from negligible at low concentrations to complete feed refusal in highly contaminated feedstuffs. Numerous animal species are affected by DON contamination. However, swine are highly sensitive to its effects. DON is rapidly absorbed from the upper gastrointestinal tract following oral exposure, with minimal subsequent metabolism. The main toxic effect of DON is decreased protein synthesis causing multiple cellular functional abnormalities that may lead to cell death. Immunosuppression caused by impaired protein synthesis can also occur in animals fed DON-contaminated feed. Increased disease susceptibility and delayed immune responses may occur with intermediate (1 to 5 mg per kg) to high concentrations (> 5 mg per kg) of DON being fed to pigs. An accurate diagnosis of acute or chronic DON ingestion in swine can be difficult. Suboptimal feed intake and growth performance and increased morbidity are clinical effects resulting from chronic exposure. In addition, DON ingestion can be obscured by other diseases resulting from its immunosuppressive effects. Testing complete feed or primary-source ingredients for DON is the most reliable way to determine exposure. However, diagnosing DON contamination by analyzing the feed from the bulk bin of apparently clinically affected animals can be challenging if the distribution of DON in the feedstuffs is intermittent or highly variable.

The objective of this assessment was to determine appropriate diagnostic tests and tissue specimens necessary to accurately diagnose chronic DON ingestion in pigs consuming 5 mg per kg DON in complete feed for 120 days.

Materials and methods

The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee. The twenty-four 11-week-old cross-bred pigs of both genders assessed in the present study were a subset of pigs used in a larger feeding trial evaluating the effects of DON and commercial mycotoxin binding agents on growth performance. Pigs were initially allocated to pens on the basis of body weight (22.9 ± 4.3 kg) and gender, and were assigned to a treatment group using a random number generator in a complete block design. Pigs included in the present study were randomly selected (by random number generator) from control pens (Control; n = 6) fed a diet that contained 0.2 to 0.7 mg per kg DON or from highly contaminated groups (DON-fed; n = 18) receiving diets containing approximately 5 mg per kg DON. Mycotoxin binders were not included in either diet during the feeding period.

Pigs were housed in a 1040-head commercial finishing facility with a computerized feeding system (Feedlogic System, Willmar, Minnesota) to deliver specific diets in measured quantities to each pen. Pens were equipped with one feeder and two nipple waterers. Pigs were fed ad libitum a corn-soy-based diet containing 20% dried distillers' grains with solubles (DDGS), with DON obtained from DDGS from two different corn ethanol plants. Highly contaminated (18.6 mg per kg DON) and clean DDGS (0.81 mg per kg DON) were procured from plants in Indiana and Iowa, respectively. Pigs were phase-fed six balanced diets according to age, with all diets containing 9 g per tonne virginiamycin (Stafac; Phibro, Ridgefield Park, New Jersey). Dietary inclusion of sodium, fed as sodium selenite, was 0.3 mg per kg of complete feed. Delivered feed samples were saved from all diets, and samples from phases two, three, and five were analyzed by liquid chromatography-tandem mass spectrometry for confirmation of DON quantities according to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) standard operating procedure.

Pigs were vaccinated with commercial vaccines for porcine circovirus type 2 and Mycoplasma hyorhinum prior to the study. Pigs were serologically positive for porcine reproductive and respiratory syndrome virus.

Serum samples were collected from Control and DON-fed pigs at the start of the study (Day 0) and at Day 117. Blood samples were allowed to clot and were chilled and centrifuged within 3 hours of collection, and sera were distributed in 5-mL aliquots for storage at -80°C. Serum analyte concentrations were measured the day of collection (Vitros 5.1 Chemistry Analyzer; Ortho Clinical Diagnostics, Johnson and Johnson, Rochester, New York). Analytes included sodium, potassium, chloride, bicarbonate, calcium, phosphorus, magnesium, blood urea nitrogen (BUN), creatinine, glucose, total protein, albumin, aspartate aminotransferase (AST), creatine kinase (CK), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), and anion gap. Globulin concentrations were calculated by subtracting albumin concentration from the total protein value.

Control and DON-fed pigs were weighed on Day 0 and again on Day 120, and then were necropsied. Macroscopic lesions were recorded by a veterinary pathologist blinded to treatment group. Tissues collected included thymus, thyroid, heart, lung, liver, gall bladder, pancreas, adrenal glands, kidneys, urinary bladder, spleen, stomach (glandular and pars esophagea regions), duodenum, jejunum, ileum, colon, bone (second rib), and tracheobronchial, mesenteric, and inguinal lymph nodes. Individual weights were recorded for the thyroid and adrenal glands. Fresh tissues were stored at -80°C.

Fresh liver samples were quantitatively assessed for concentrations of cadmium, calcium, cobalt, copper, chromium, iron, phosphorus, potassium, magnesium, manganese, molybdenum, selenium, and zinc by inductively coupled plasma-mass spectrometry (ICP-MS) (ICP mass spectrometer; Varian, Santa Clara, California) according to the ISU-VDL standard operating procedure. Briefly, two 1-gram samples were weighed into Teflon vessels (MARSXpress TFM digestion vessels; CEM Corporation, Matthews, North Carolina) with 2 mL of 18 MΩ water (Aries 1105D, Direct Feed Laboratory Water System; Aries filter works, a division of Resintech, Inc, West Berlin, New Jersey) followed by 10 mL of trace-metal grade concentrated nitric acid. Vessels were sealed, vortexed, and subsequently microwaved to digest the sample. After cooling, samples were filtered (Whatman #40 filter paper, ashless, circles, 90 mm; Whatman Inc, Piscataway, New Jersey) and diluted to 25 mL with 18 MΩ water. Filtered samples were diluted 1:10 in 1% nitric acid for calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc assays, and were diluted 1:3 for cadmium, chromium, cobalt, molybdenum, and selenium assays. Samples were analyzed with internal standards and control liver that bracketed the concentration range of each analyte.
Bone ash and density were determined from the second rib according to the ISU-VDL standard operating procedure. Soft tissue, periosteum, and the distal costochondral portion were discarded. A 5-cm section of the remaining rib was removed and tested. Individual samples were dried, weighed, placed in a beaker of water, and vacuum sealed overnight to remove air bubbles. The following day, the weight of the bone suspended in water was recorded. Density was calculated using Archimedes’ principle. Bone ash was determined by recording the weight of the second rib used for the density measurement prior to placement in a muffle oven overnight at 500°C. Percent bone ash was calculated by dividing the weight of the bone ash by the weight of the original bone.9

Formalin-fixed tissues were paraffin-embedded, sectioned, stained with hematoxylin and eosin, and evaluated by a veterinary pathologist blinded to treatment group. Microscopic lesions were assessed in all tissue sections. Stomach, small intestine, and colon were individually scored for the amount of inflammation in the mucosa and the submucosa (0, normal; 1, mild; 2, moderate; 3, marked) and for the amount of gut-associated lymphoid tissue (GALT) hyperplasia (0, none; 1, mild; 2, moderate; 3, marked). An average score for the small intestine was calculated using the individual scores from five different sections evaluated. A total score was calculated for stomach, small intestine, and colon by combining the mean values for mucosa, submucosa, and GALT with possible scores ranging from 0 to 9.

Summary statistics (JMP software version 8.0.2; SAS Institute, Cary, North Carolina) were calculated for all groups to assess the overall quality of the data set. A two-tailed Student t test was used for mean comparisons between treatment groups. For serum chemistry values, analysis of variance (ANOVA) was conducted with an effects test to measure the effect of time. Differences were considered statistically significant at P < .05.

Results

Growth rate, as measured by average daily gain (ADG), did not differ between Control pigs (0.79 ± 0.02 kg) and DON-fed pigs (0.77 ± 0.01 kg) evaluated in the present study (P > .05). Few macroscopic lesions were apparent at necropsy. Mild chronic fibrosing pleuritis with adhesions to the thoracic cavity was observed in one Control pig and two DON-fed pigs. The DON-fed group included one pig with chronic pericardial fibrosis and one pig with mild fibrosing peritonitis. All other organs and organ systems appeared normal in both groups.

Serum chemistry values at Days 0 and 117 did not differ between the Control and DON-fed groups (P > .05). However, a significant effect of date (age) was observed between Day 0 and Day 117 for several analytes that decreased with age, including phosphorus (P < .001), potassium (P < .001), magnesium (P < .001), and AST (P < .001), while glucose (P = .02), BUN (P < .001), and CK (P < .01) increased (data not shown).

Liver analyte values are reported in Table 1. Liver selenium concentrations (reference interval 0.40 to 1.20 mg per kg10) were significantly lower (P = .02) in the DON-fed group than in the Control pigs. Adrenal gland weight and thyroid weight as percentages of body weight, bone ash percentage, and bone density did not differ between treatment groups (Table 2).

Microscopic lesions were not evident in examined sections of bone, heart, lymph nodes, pancreas, spleen, thymus, thyroid, or urinary bladder in either group. Sporadic inflammatory lesions were noted in both groups and included interstitial pneumonia, interstitial nephritis, scant lymphocytic adrenalitis, and mild cholecytitis. Neither hepatocellular necrosis nor vacuolar degeneration was observed in the sections examined in either group.

Gastrointestinal lesion scores did not differ between Control and DON-fed pigs. Mean microscopic lesion scores for stomach, small intestine, and colon in Control pigs were 2.67, 1.20, and 2.83, respectively. Mean scores for DON-fed pigs were 3.34, 1.17, and 3.61 for stomach, small intestine, and colon, respectively. The pars esophagea region of the stomach, which included squamous and non-squamous areas, contained moderate to marked GALT hyperplasia in three Control and 11 DON-fed pigs. These nodular lymphocytic aggregates were present within the lamina propria and sometimes extended into the submucosa. Epithelial erosions or ulcerations were not identified.

Discussion

Each year, approximately 25% of cereal grains produced globally are contaminated with DON.11 DON is more abundant in grains harvested in regions with high humidity and cool temperatures and is further amplified by storing grains contaminated with Fusarium species at high moisture levels.1 In 2009, high atmospheric humidity and abnormally cool temperatures during corn harvest in the US corn belt resulted in soaring DON concentrations, which were subsequently fed in large amounts to production livestock. Further compounding the issue for some livestock producers was the inclusion of DDGS in the diet. Fermentation of corn to ethanol does not degrade mycotoxins, but amplifies their concentration three-fold in the finished corn by-product.12

The predominant clinical effects of feeding high concentrations of DON to pigs are reduced feed intake or refusal, resulting in suboptimal growth performance. In the larger study from which these pigs were derived, pigs receiving highly contaminated DON feed had significantly lower ADG than did pigs receiving feed that contained only 0.2 to 0.7 mg per kg DON.8 Differences in ADG were not detected in the subset pigs evaluated in this study, likely due to the smaller sample size of the Control pigs. Vomiting, occasional diarrhea, and the potential for rectal prolapse has been documented after feeding DON.3 Furthermore, smaller thyroid size and squamous hyperplasia of the pars esophagea region were reported in swine fed increasing levels of DON (up to 3 mg per kg) for 28 days, compared to pigs without DON-contaminated feed.13 In contrast, the results in the present study did not detect significant differences in thyroid weights between groups after 120 days, and squamous hyperplasia in the stomach was not evident. However, pigs can adapt to DON ingestion.14 Therefore, the lack of significant differences between the groups described in this study may be due to pigs acclimating to high levels of DON in the diet with subsequent resolution of lesions over time.

Other investigators have evaluated organ weights after feeding different levels of DON. Reports do not agree and fail to account for differences in kidney, liver, and spleen weights relative to body weight. Additional reports have demonstrated higher relative liver and kidney weights with DON ingestion.13,15-17 Individual liver, spleen, and kidney weights were not evaluated in this study. However, adrenal glands were weighed as a potential determinant of reduced protein synthesis caused by feeding elevated DON concentrations. Under the conditions of this assessment, significant differences in adrenal weights were not detected.
Consistent with previous reports, serum analyte values did not differ between groups in the study described here. Alternatively, others have described lower serum calcium, phosphorus, cholesterol, ALP, total protein, and globulin, and higher serum chloride, ALP, and albumin in pigs ingesting higher concentrations of DON than controls. Serum analyte changes noted in previous reports may be due to feeding DON for periods of time shorter than those used in this study. The authors acknowledge that analyte values may have varied throughout the feeding period. From a diagnostic perspective, serum analyte values are highly variable and may be dependent on both the duration and dose of DON ingestion.

Inductively coupled plasma-mass spectrometry, bone ash, bone density, and liver analysis may be used to assess the long-term effects of DON ingestion and how it alters the storage of different analytes. To the authors’ knowledge, these methods have not been reported for pigs being fed DON-contaminated feed for extended periods of time. Selenium was the only analyte that was significantly lower in the DON-fed group. DON ingestion has multiple effects on cellular functions, including decreased protein synthesis and lipid peroxidation. Selenium is a well-known antioxidant as a component of superoxide dismutase and...
is important for both immunity and cell survival through limiting the effects of lipid peroxidation.\textsuperscript{25,26} Lower liver selenium concentrations in DON-fed pigs were not unexpected, but to the authors' knowledge, this has not been previously reported. Supplementing with higher levels of selenium in DON-contaminated feed decreases liver lipid peroxidation in rats\textsuperscript{27} and reversed the adverse effects on the immune system in chickens.\textsuperscript{28} These results raised speculation that increased antioxidants in swine diets known to be contaminated with DON may reduce the physiological effects associated with ingestion. Bone ash and density did not differ between groups. Under the conditions of this study, these results suggest that DON has no physiological effect on bone growth or bone integrity and further confirms that serum calcium and phosphorus disturbances measured in other reports may be transient or unrelated to DON ingestion.

Few studies have evaluated microscopic lesions associated with feeding DON. Similar to other measured parameters, inconsistent microscopic lesions are likely associated with dose and timing of sampling the animals after DON ingestion. Previous studies have reported variations in the presence of microscopic lesions ranging from none detected\textsuperscript{16} to lesions that were considered relevant to DON ingestion.\textsuperscript{29} Recently, it was documented that intravenous administration of DON to pigs results in lymphocyte apoptosis in multiple lymphoid organs and other tissues, with more severe lesions observed at 24 hours. Hepatocellular apoptosis has also been reported.\textsuperscript{30} Following a 6-week feeding study with DON-contaminated feed, lymphocytic depletion was apparent in the spleen, and hepatic vascular dilatation and thickening and focal hemorrhagic and necrotizing lymphadenitis were reported.\textsuperscript{22} Two other groups observed an increase in hepatic fibrosis separating lobules.\textsuperscript{15,31} However, both studies had mixed mycotoxins in the feed, including DON and aflatoxin and DON and zearalenone contamination, respectively. The complete feed used in this study contained minimal quantities of zearalenone (0 to 0.6 mg per kg) and fumonisin B1 (0 to 1.1 mg per kg), but not aflatoxin.\textsuperscript{8} These concentrations are considered within normal limits in swine feed. Evidence of previous or persistent hepatic degeneration or necrosis was not observed. These data suggest that liver histopathology cannot be reliably used to diagnose chronic DON ingestion. An important aspect of this study was its extensive evaluation of the gastrointestinal tract for microscopic lesions that may be linked to DON ingestion. Feeding diets contaminated with DON alone has been demonstrated to cause mild villous atrophy, edema, and hyperemia during the acute post-feeding period.\textsuperscript{32} Feeding diets with multiple mycotoxin contaminants (DON, T-2 toxin, and zearalenone) resulted in mild inflammation, goblet cell loss, and increased crypt necrosis.\textsuperscript{33} A study in mice fed DON and nivalenol reported inflammation and crypt necrosis of the small intestine, and gastric ulceration and inflammation.\textsuperscript{34} Furthermore, DON can alter intestinal microflora by increasing aerobic mesophilic bacteria.\textsuperscript{35} DON consumption has also been shown to promote uptake of Salmonella serovar Typhimurium by macrophages, suggesting there may be an increased susceptibility to gastrointestinal pathogens in swine.\textsuperscript{7} For these reasons, in this study, an intestinal inflammatory score was recorded along with morphological changes. Diagnostic changes associated with abnormal villi, loss of goblet cells, crypt changes including necrosis or increased mitoses, or colonic glandular changes were not apparent in examined sections of small and large intestine. These data suggest that either higher doses of DON may be needed to cause gastrointestinal inflammation, or pigs chronically fed DON physiologically adapt with time.

Evaluating swine tissues macroscopically and microscopically, performing bone analysis, or measuring serum analytes (including liver enzyme activity) demonstrated limited diagnostic value in determining chronic DON ingestion in pigs fed 5 mg per kg DON in complete feed for 120 days. Alternatively, these data suggest that measuring liver selenium concentrations may aid in the diagnosis of chronic DON exposure in pigs.

**Implications**

- Under the conditions of this study, chronic DON ingestion does not cause significant macroscopic or microscopic tissue lesions.
- Under the conditions of this study, in pigs fed 5 mg per kg DON in complete feed for 120 days, bone and serum analyte analysis are of limited diagnostic value.
- Liver trace-mineral analysis may provide a useful diagnostic tool suggesting chronic DON ingestion.
- Additional studies are necessary to evaluate the effects of chronic DON ingestion in pigs fed concentrations of DON > 5 mg per kg of feed.

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**Conflict of interest**

None reported.

**References**


