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B. Rajasekhar Reddy
N. Sethunathan*

Laboratory of Soil Microbiology
Central Rice Research Institute
Cuttack 753006, India

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Nontransmission of Deoxynivalenol (Vomitoxin) to Eggs and Meat in Chickens Fed Deoxynivalenol-Contaminated Diets

Deoxynivalenol (DON, vomitoxin) present in the rations of Leghorn chicks and laying hens and broiler chickens at dietary levels of 4–5 μg/g for periods of between 28 and 190 days was not detected in eggs or tissues (drumstick, breast, liver, and gizzard). The detection limit of the method used was 10 μg of DON/kg of tissue. It was also determined that DON was stable in feed (kept at room temperature, ≥10 °C), eggs (23 °C), and chicken tissue (4 °C).

Considerable evidence has now accumulated on the natural occurrence of vomitoxin [3α, 7α, 15-trihydroxy-12,13-epoxytrichothec-9-en-8-one; deoxynivalenol (DON)] in grains grown in many parts of the world (Scott et al., 1981; Trenholm et al., 1981; Ueno, 1980; Vesonder and Hesseltine, 1981). DON is a metabolite of *Fusarium graminearum* (perfect stage *Gibberella zeae*) that has been found to be an emetic agent in swine, dogs, and ducklings (Ueno, 1980), to cause feed refusal by swine and rats (Forsyth et al., 1977; Yoshizawa et al., 1978), and to be teratogenic in mice (Khera et al., 1982). Poultry, however, apear to be relatively insensitive to DON (Hamilton et al., 1981a,b; Huff et al., 1981; Hulan and Proudfoot, 1982; Moran et al., 1982), which raises the possibility that DON-contaminated feed grains may be incorporated into rations of laying hens and broiler chickens without affecting performance. Therefore, it is important from the human health viewpoint to establish whether DON residues occur in eggs and tissues when poultry are given rations that contain DON. This study was undertaken to answer this question when DON naturally present in wheat was fed to White Leghorn chicks and laying hens and broiler chickens at levels about 4–5 μg/g of feed over continuous periods of between 28 and 190 days (Hamilton et al., 1983). No previous residue studies on DON in any animal species have been published. However, the transmission of other polar mycotoxins (ochratoxin A and aflatoxins) to eggs and tissues of chickens fed contaminated feed has been reported by several investigators (Harwig et al., 1983; Juszkiewicz et al., 1982; Rodricks and Stoloff, 1977).

EXPERIMENTAL SECTION

Feed Samples. Spring wheat was the major component of the control and DON-containing rations that were otherwise nutritionally balanced for laying hens, Leghorn chicks, and broiler chickens. These rations, whose major source of protein was a 48% protein-containing soybean meal, were formulated to be isocaloric as well as isonitrogenous because the protein content of the DON-contaminated wheat was much higher than that of the noncontaminated wheat (13.6 vs. 9.8%, N × 5.8, respectively). Therefore, the contaminated and noncontaminated rations contained 51–66% and 57–74% wheat, respectively, depending on the type of ratio prepared. Chemical analyses (Laboratory Services Division, Agriculture Canada) indicated that the noncontaminated wheat contained <0.05 μg of DON/g while the contaminated wheat contained 7.6 μg of DON/g. Sufficient quantities of the Leghorn chick and broiler chicken rations were mixed at one time to last throughout the experimental period, while approximately a 3-month supply of the laying hens rations were mixed at one time. Calculated DON concentrations in the contaminated diets were about 5 μg/g for the laying hens rations and Leghorn chick and broiler chicken starter rations and about 5.5 μg/g for the broiler chicken finisher rations. Samples of all rations were collected at the time of mixing for chemical analyses and were subsequently stored at −20 °C. The remainder of the rations were stored at room temperature (≥10 °C) until they were fed to the birds in the form of a mash.

Laying Hens: Eggs and Tissues. Two sets of eggs were collected from Leghorn hens that had been fed ad libitum contaminated or control laying rations for 146 and 160 days, respectively. Each set contained equal numbers of eggs from the hens given each of the two diets. After having been fed the aforementioned diets for 168 or 190 days, randomly selected hens were killed by carbon dioxide inhalation between 8.00 and 10.00 a.m. and samples of breast muscle, drumstick, liver and gizzard were collected and immediately frozen. The hens were 361 days of age at the beginning of the experimental period.

Chicks: Tissues. White Leghorn chicks and broiler chickens were killed as described previously, and breast

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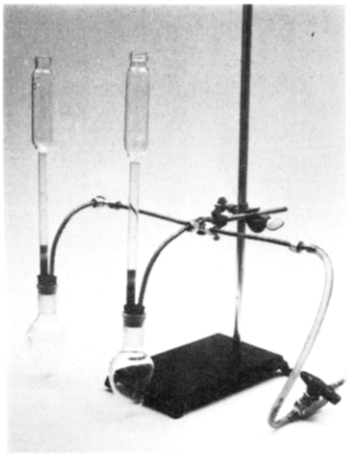

Figure 1. Alumina–charcoal columns used for extract cleanup.

muscle, drumstick, thigh (chicks), liver, and gizzard (broiler chickens) samples were collected and frozen immediately. Both types of chicks were fed ad libitum either a control ration that contained no measurable DON or one containing DON. Tissues were collected from 9 or 10 Leghorn chicks and 12 broiler chickens that received each of the rations. For the Leghorn chicks, starter rations were fed from 7 days until the time of necropsy at 35 days of age. The broiler chickens were given starter rations from 7 to 28 days and finisher rations from 29 to necropsy at 55 days of age.

Preparation of Samples for Analysis. Eggs were kept at 4 °C and the animal tissue samples were kept frozen until analysis (maximum 10 days). Each egg sample was prepared by blending three to four whole eggs for 1 min in a Waring Blendor at low speed just before analysis. Tissue samples were prepared by mincing the tissues from five to six birds twice in a food mincer. In the case of the drumsticks, they were first deboned and the tissues were subsequently minced.

Preparation of Alumina–Charcoal Cleanup Column (Romer et al., 1981). A chromatographic glass column 18 cm × 1.1 cm o.d. with a 10 cm × 2.9 cm o.d. reservoir and no stopcock (Kontes Scientific Glassware) was used (Figure 1). Darco G-60 decolorizing carbon (0.75 g) was added to a Pyrex glass wool plug (0.15 g) at the bottom of the column, using suction to compact the layer. Bio-Rad AG-7 neutral alumina, 100–200 mesh (0.70 g) was then added and the column was capped with glass wool (0.15 g).

Spiking of Samples. For recovery experiments, 50-g samples of whole egg were spiked with 60 μL of stock solution prepared from crystalline DON (250 μg/mL of acetonitrile or chloroform). Chicken breast muscle, drumstick muscle, liver, or gizzard were spiked at two levels: 0.3 and 0.15 μg/g using 60 μL or 30 μL of the stock solution per 50-g sample, respectively.

Extraction. Each 50-g whole egg sample was shaken for 30 min in a 500-mL glass-stoppered Erlenmeyer flask with 185 mL of acetonitrile to give an acetonitrile:water ratio of ca. 84:16 (Romer et al., 1981), assuming that the water content of whole egg is 70%. Each 50-g chicken sample was blended for 5 min at highest speed in a blender (Osterizer) with 195 mL of acetonitrile (assuming that the moisture content of the tissue is 74%). Feed samples (50 g) were blended for 5 min with 200 mL of acetonitrile-water (84:16). The mixture in each case was filtered through Whatman No. 4 fluted paper.

Cleanup. The method of Romer et al. (1981, 1982) was used with the following modifications. Five milliliters of filtrate was added to the alumina–charcoal cleanup column and was eluted under reduced pressure as illustrated in Figure 1. An additional 5 mL of acetonitrile–water (84:16) was used to wash the column. The combined eluates were evaporated to dryness by using a rotary evaporator at 50 °C, and the residue was dissolved in toluene–acetonitrile (95:5) to give concentrations of 1 g of sample/mL for egg or chicken tissue samples and 0.1 g of sample/mL for feed samples.

Determination of DON. Gas–liquid chromatography (GLC) was carried out after derivatization with (heptafluorobutyryl)imidazole (HFBI) as described by Scott et al. (1981). The final concentration of egg or chicken tissue samples in the diluted derivatized extract was equivalent to 100 mg/mL and an equivalent to 5 mg/mL was used for the feed samples. The concentration of DON in the diluted derivatized standard was 25 ng/mL. Estimations were made in duplicate by comparison of peak heights in the injected sample with standard injected before and after the sample using GLC operating conditions used previously (El-Banna et al., 1983). GLC–mass spectrometric single ion monitoring [MS(SIM)] of DON tris(heptafluoro-

Table 1. Amount of Deoxynivalenol (DON) Recovered from Egg, Chicken, and Feed Samples

samples	experiment 1 DON, μg[a]	experiment 1 recovery, %	experiment 2 DON, μg[a]	experiment 2 recovery, %
egg	14.5[b]	96.7	12.2[b]	81.3
chicken liver	15.9[b]	106	6.87[c]	91.6
chicken breast	15.0[b]	100	6.52[c]	86.9
chicken gizzard	14.0[b]	93.3	5.81[c]	77.5
chicken drumstick	6.7[c]	89.3	6.64[c]	88.5
feed	175.8[d,e]	87.9	182.3[d,f]	91.2

[a] Samples not spiked contained no detectable DON (less than 10 μg/kg). [b] Amount of DON added to each 50-g sample was 15 μg. [c] Amount of DON added to each 50-g sample was 7.5 μg. [d] Amount of DON added to each 50-g sample was 200 μg. [e] Laying hen ration. [f] Broiler finisher ration.

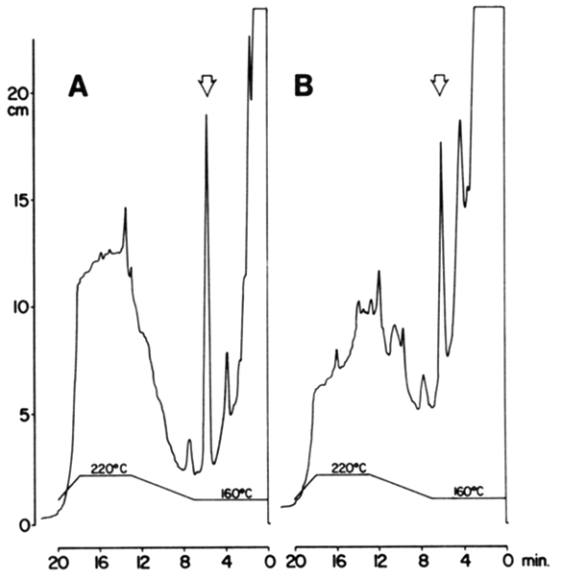

Figure 2. GLC of extracts from the drumstick muscle of chickens and eggs spiked with 0.15 and 0.3 μg of DON/g, respectively. Arrows indicate the retention time of DON tris(heptafluorobutyrate): (A) 1.4 μL of 100 mg equiv of egg/mL injected and estimated to contain 40.6 pg of DON; (B) 2 μL of 100 mg equiv of chicken muscle/mL injected and estimated to contain 26.5 pg of DON.

Table II. Stability of Deoxynivalenol (DON) in Chicken Meat (Drumstick) during Storage at 4 °C[a]

storage time, days	DON, μg[b]	DON remaining, %
0	6.74	100
1	6.64	98.5
5	6.95	103.1
6	5.47	81.2

[a] Amount of DON added to each 50-g sample at zero time was 7.5 μg. [b] In 50 g of chicken meat, uncorrected for recovery.

Table III. Stability of DON in Feeds of Laying Hens and Broiler Chickens

sample	storage time, weeks	storage conditions, °C	DON, μg/g[a]
laying hens feed			
control	0.0		ND[b]
	19	≥10	ND
	19	−20	ND
DON contaminated	0.0		5.2[c]
	19	≥10	3.89[d]
	19	−20	3.87[d]
broiler finisher feed			
control	0.0		ND
	5	−20	ND
DON contaminated	0.0		5.4[c]
	5	−20	5.11[d]

[a] Corrected for recoveries (see Table I). [b] No detectable amount (less than 10 μg/kg). [c] Calculated values based on the measured DON content of the wheat used to prepare the diets. DON content of the wheat was measured by Laboratory Services Division, Agriculture Canada. [d] Duplicate determinations, differing by 3.5–26% (one analysis carried out on a 1% SE-30 column at 180 °C, Varian 2100 instrument).

butyrate) molecular ion at m/z 884 was also done as described by El-Banna et al. (1983).

Stability of DON. One hundred fifty grams of egg was spiked with DON at a concentration of 0.3 μg/g. Fifty grams was analyzed 0.5 h after being spiked, and the rest was left at room temperature for 24 h before analysis of a second 50-g sample. Drumsticks of broiler chickens that showed no detectable DON (control) were deboned, minced, and divided into 50-g portions in separate blender jars. After spiking with DON (0.15 μg/g), one sample was analyzed for DON and the rest were stored at 4 °C for 1, 5, or 6 days before analysis. Samples of the laying hen ration were stored about 19 weeks at the ambient temperature of the storage room (≥10 °C) or in the freezer (−20 °C). Samples of broiler finisher diets were also analyzed after 5 weeks of storage at −20 °C.

RESULTS AND DISCUSSION

The average recovery of DON from egg was 89% at a concentration of 0.3 μg/g, while recoveries of DON from different types of chicken tissue ranged from 93–106 and 77–92% at 0.3 and 0.15 μg/g spiking levels, respectively (Table I; Figure 2). The method used in this study is simple, interferences from sample impurities are minimal, and the minimum detectable amount of DON was estimated to be about 1 pg. This method appears to be very promising for detection and determination of DON in foods of animal origin and is the first for DON in these products to be described.

A total of 52 samples of egg and chicken tissues were analyzed by GLC (detection limit 10 μg/kg, based on a prorated peak height of 0.5 cm) for detection and determination of DON. These samples were 4 egg samples, 12 samples of tissues from Leghorn chicks (4 each of breast, liver, and drumstick/thigh), and 16 and 20 samples from broiler chickens and laying hens, respectively, representing breast, liver, drumstick, and gizzard tissues. Equal numbers of samples of eggs and each tissue were obtained from birds fed DON-contaminated feed and those fed DON-free feed. None of these samples were found to contain detectable quantities of the toxin, which demonstrates that the DON from the ration was not transferred to the eggs and tissues of Leghorn chicks and laying hens and broiler chickens fed DON-contaminated feed. These negative results were confirmed by GLC–MS(SIM) for six tissue samples from chicks (including three controls) and five samples from laying hens (including two controls); the detection limit was 5 μg/kg (signal:noise = 3:1).

Comparison of these results with those obtained with other trichothecenes is of interest. No residues of T-2 toxin [3α-hydroxy-4β,15-diacetoxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-ene] could be detected in liver and muscle tissue of broiler chickens fed 15 μg of T-2 toxin/g of diet for 43 days (Hofmann, 1980). However, T-2 toxin and HT-2 toxin [3α,4β-dihydroxy-15-acetoxy-8α-[(3-methylbutyryl)oxy]-12,13-epoxytrichothec-9-ene] were detected for up to 5 h in livers of roosters that had rapidly ingested about 1.2 mg of T-2 toxin (Hofmann, 1980). Chi et al. (1978a) showed that the equivalent of 0.9 μg of tritium-labeled T-2 toxin was transmitted into an egg by laying hens intubated daily with 1 mg of tritium-labeled T-2 toxin/kg of body weight for 8 days. They also found that the muscle of chicks contained 0.06 and 0.04 μg of T-2 equivalent/g of wet tissue at 24 and 48 h, respectively, after dosing with 0.5 mg of tritium-labeled T-2 toxin/kg of body weight and there were 0.1 and 0.04 μg/g in the liver (Chi et al., 1978b).

Deoxynivalenol was found to be stable in chicken meat (drumstick) during storage at 4 °C for 6 days (Table II). The stability of DON in eggs during storage at room temperature for 24 h was also studied and the amount of DON remaining was 97.6%. The stability of DON in feed kept at ≥10 °C compared to feed kept at −20 °C is shown in Table III. These supplementary data given additional validity to our findings of nontransmission of DON to eggs and chicken tissues. However, the possibility that metabolites may be present could be easily demonstrated by further experiments using radioactively labeled DON.

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Amr A. El-Banna[1,3]
Robert M. G. Hamilton[2]
Peter M. Scott*[1]
H. Locksley Trenholm[2]

[1]Food Research Division
Health Protection Branch
Health and Welfare Canada
Ottawa, Ontario, Canada, K1A 0L2
[2]Animal Research Centre
Agriculture Canada
Ottawa, Ontario, Canada K1A 0C6
[3]Visiting scholar from the Department of Agricultural Industries
Faculty of Agriculture
University of Alexandria
Alexandria, Egypt

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Positive Identification of New Alkyloxazoles, Alkylthiazoles, and Piperidine in Roasted Peanut Flavor

Previous work provided evidence of the occurrence of new alkyloxazoles, alkythiazoles, and 2-(2-aminoethyl)piperidine in the volatiles of freshly roasted peanuts. Here the synthesis and mass spectral data of these compounds are reported.

Roasted peanuts possess a unique and widely enjoyed flavor. There are more than 350 volatile compounds identified in roasted peanuts (Buckholz and Daun, 1981). Recently, we reported the identification of 131 compounds in the volatile flavor constituents of freshly roasted peanuts (Ho et al., 1982).

Thiazoles and oxazoles have recently been recognized as important flavor components in food. Walradt et al. (1971) identified thiazole, 4-methylthiazole, and benzothiazole in the volatiles of roasted peanuts. Buckholz and Daun (1981) reported the identification of 2,4-dimethyl-3-thiazoline in roasted peanut flavor and found that it added deep roasted notes and contributed to the nut skin character. We reported the identification of eight thiazoles, seven oxazoles, and three oxazolines in roasted peanut flavor (Lee et al., 1981). However, due to the lack of reference mass spectral data, several alkylthiazoles, alkyloxazoles, and a piperidine derivative were only tentatively identified (Lee, 1980).

In the present paper, we report the synthesis and mass spectral data of six oxazoles, seven thiazoles, and 2-(2-aminoethyl)piperidine.

EXPERIMENTAL SECTION

Synthesis of α-Bromo Ketones. α-Bromo ketones were synthesized by the method described by Catch et al. (1948), which involves essentially direct bromination of the appropriate ketone. With unsymmetrical ketones, two bromo ketones are formed. In all cases, the mixture of the two bromides was taken through the oxazole synthesis and the two isomeric oxazoles were separated by GC.

Synthesis of Alkyloxazoles. These were all synthesized by the method of Theilig (1953). One equivalent of α-bromo ketone was allowed to react with 2 equiv of amide. Yields were all generally quite satisfactory at about 50%. The distilled products were purified by GC.

Synthesis of Alkylthiazoles. Alkylthiazoles were synthesized by the method of Kurkjy and Brown (1952) by the addition of the α-bromo ketone to the preformed thioamide. Yields were all quite satisfactory at about 50%. The steam distilled products were then purified by GC.

Synthesis of 2-(2-Aminoethyl)piperidine. To 5 g of 2-(2-aminoethyl)pyridine (Aldrich Chemical Co.) in 100 mL of diethyl ether dry hydrogen chloride gas was intro-

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