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Absorption and Elimination of an Oral Dose of ^{3}H-Deoxynivalenol in Colostomized and Intact Chickens

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Deoxynivalenol (DON or 3, 7, 15-trihydroxy-12, 13-epoxy-trichothec-9-en-8-one) is a mycotoxin produced by Fusarium graminearum that can contaminate grain. Domestic fowl are particularly tolerant to DON ingestion (Moran et al. 1982; Trenholm et al. 1984; Hamilton et al. 1985). DON per se disappears from the upper small intestine and little appears in the excreta of hens (Lun et al. 1988). Prelusky et al. (1986) orally administered ^{14}C-DON to chickens and observed high radioactivity in the liver and bile with over 90% of the original label accruing in the excreta before 48 h.

DON cannot be detected in portal blood concurrent to its disappearance from the gastrointestinal tract (GIT) (Lun et al. 1988). Presumably, DON was structurally modified upon absorption then hepatically retrieved and excreted in bile as T-2 is suspected of doing (Chi et al. 1978). In the present experimentation, ^{3}H-DON was intubated into colostomized and intact hens. The objective was to measure the progressive changes in distribution of radioactivity along the GIT, among body tissues and between urine and feces.

MATERIALS AND METHODS

^{3}H-Don was prepared according to Wallace et al. (1977). Essentially, 50 ul of dimethyl sulfide was added to 44 mg of N-chlorosuccinimide in 5 ml of dry toluene at 0°C under dry N_2. The mixture was cooled to -25°C then 30 mg DON in dry toluene were added. After 2 h, 35 mg triethylamine in .2 ml dry toluene was reacted with the mixture for 5 min at room temperature. The resulting oxidized DON was extracted with 5x20 ml ethylacetate and the composite washed once with .1N HCl and twice with 15 ml of H_2O. After evaporative removal of the

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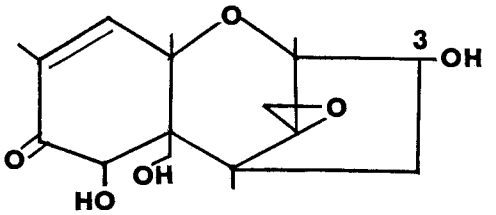

Figure 1. Structure of DON. The H at the number 3 C is displaced upon oxidation of the associated hyroxyl group to a ketone and replaced with ^3H during subsequent borohydride reduction.

solvent (40°C), the residue was dissolved in 1 ml isopropanol and transferred to a dram vial containing 25 mCi of ^3H-sodium borohydride. The subsequent reaction incorporated a nonionizable ^3H label at C-3 as the oxidized DON was reduced to its original form. After 15 min at room temperature, 15 ml of ethylacetate was added, and the mixture was washed once with 3 ml .3N HCl. The aqueous layer was extracted of its ^3H-DON with 3x10 ml ethylacetate after which the solvent was evaporatively removed.

^3H-DON contained in the residue was dissolved in 200 ul of acetonitrile and purified twice by TLC on silica G plates using $CHCl_3$-MeOH-H_2O (90:10:1 v/v) and $CHCl_3$-CH_3COCH_3 (3:2 v/v) solvent systems, respectively. Radioactivity of the purified DON was measured in a Packard Tricarb liquid scintillation spectrometer (Model 3375). The GC method previously described by Lun et al. (1986) was used to measure DON concentration. Recovery of DON in total was 8.7% with a specific radioactivity of 2.2 mCi/mmol.

Stability of the label was tested in acid conditions similar to that in the lumen of the gizzard. A sample of ^3H-DON was incubated in .075N HCl (pH 1.03) for 2 h at 41°C, then the mixture was cooled to room temperature and extracted 5x with two equal volumes of ethyl acetate. The solvent composite was evaporated to dryness, reconstituted in acetonitrile and purified by TLC using the $CHCl_3$-MeOH-H_2O (90:10:1 v/v) system. Recovery of radioactivity relative to total DON was 87%.

Experiment 1 used three actively laying Single Comb White Leghorn (SCWL) hens at 32 weeks of age which had been colostomized by exteriorization of the distal colon through the musculature of the left flank. These birds were individually maintained in metabolism cages

Table 1. Composition of DON contained feed

Ingredient	%	Analysis	%
Control corn	66.97	Crude protein	17.32
Contaminated corn	2.00	Ether extract	2.44
Soybean meal (48% PC)	21.00	Crude fiber	2.56
Dicalcium phosphate	1.50	Calcium	3.47
Limestone	7.50	Phosphorus	.73
DL-methionine	.03	(DON ppm	5.80)
Iodized salt	.25	(Zearalenone ppm	.46)
Vitamin mix[1]	.50		
Mineral mix[2]	.25		
Total	100.00		

[1]Supplied the following per kg of feed: vit. A 8000 IU; vit. D_3 1600 IU; vit. E 11 IU; riboflavin 7 mg; d-Ca pantothenate 7 mg; vit. B_{12} 8 ug; niacin 20 mg; choline chloride 900 mg; vit. K 1.5 mg; folic acid 1.5 mg; biotin .2 mg; ethoxyquin 125 mg.
[2]Supplied the following in mg/kg feed: Mn 55 (MgO); Zn 50 (ZnO); Cu 5 ($CuSO_4$); Fe 30 (FeO).

and had been receiving a DON contaminated feed (Table 1) together with 24 h lighting for 1 week prior to experimentation. At the start of experimentation, each bird was crop intubated with a single dose of ^{3}H-DON (.1 mg/kg body wt; 1.64×10^6 dpm/kg) dissolved in .5 ml of 25% EtOH. Blood was taken from the brachial vein at 0, 1, 3, 6, 12, 24 and 48 h post-intubation with heparinized syringes. Urine and feces voided between each bleeding were collected and frozen (-18°C). Access to feed and water was continuous.

Experiment 2 used 18 intact SCWL hens at 33 weeks of age which were kept and dosed with ^{3}H-DON as those in Experiment 1. Three birds were sequentially killed by cervical dislocation at 1, 3, 6, 12, 14 and 48 h post-intubation. Blood samples were taken from the brachial vein before death and breast muscle, thigh muscle, abdominal fat, heart, kidneys, liver and bile samples followed. The GIT was ligated such that lumenal contents of the crop, proventriculus - gizzard, duodenum - jejunum, ileum, colon-ceca-cloaca could be quantitatively collected. Eggs produced were separated into yolk and albumen and stored frozen (-18°C) with all other tissues and excreta until analyses.

Radioactivity measurements employed the same scintillation spectrometer as described earlier. Contents from the GIT and excreta were homogenized with four parts of water (v/v) while egg yolks were homogenized with one

Table 2. Blood and excreta radioactivity after ^{3}H-DON intubation of colostomized hens[1]

Tissue	Hours after administration					
	1	3	6	12	24	48
	(% of total administered H^{3}-DON)					
Whole blood	2.69±1.48	1.84±1.37	1.17± .74	.67± .43	.45±.29	.33±.21
Plasma	1.66± .54	.92± .15	.73± .23	.19± .15	.22±.13	.15±.10
Urine	15.08±8.50	26.10±7.99	18.26±11.36	6.94±3.27	1.55±.64	1.08±.68
Feces	.29± .18	.26± .19	1.81± 1.48	2.26± .44	1.05±.64	1.14±.51

[1]All values are the average ± standard deviation of three hens each given 1.6×10^{6} dpm ^{3}H-DON/kg body weight.

part. Albumen was not diluted. Samples of homogenates of the respective tissues (100 mg) and blood (100 ul) were digested in perchloric acid and H_2O_2 (Mahin and Lofberg, 1966). Corresponding samples from birds not given ^{3}H-DON were used to correct for background counts. Quenching was corrected by the external method, and the presence of $^{3}H_2O$ was estimated from the difference in radioactivity before and after lyophilization.

RESULTS AND DISCUSSION

Administering ^{3}H-DON to colostomized hens led to the overwhelming amount of radioactivity being excreted in the urine as compared to feces (Table 2). The greatest proportion appeared between 1 and 3 h after dosing with over 75% of the total occurring by 24 h. Counts in blood samples were low and changed with time such that the highest level preceeded appearance in the urine; thus, removal by the kidneys was sufficient to keep pace with GIT absorption.

Distribution of radioactivity along the GIT of intact hens with time after intubation agreed with results from earlier experimentation involving direct measurement of DON (Lun et al. 1988). Once leaving the crop, both label and DON rapidly disappeared with little being detected in the last half of the small intestine (Table 3). Although Lun et al. (1988) failed to detect any sizable amount of DON in the large intestine or excreta, present experimentation measured substantial amounts of label between 3 and 24 h concurrent with urinary elimination from colostomized birds. Retrograde movement of urine from the cloaca into colon

Table 3. GIT, body tissue and excreta radioactivity after intubaction of intact hens with ^{3}H-DON[1]

Tissues	Hours after administration					
	1	3	6	12	24	48
	(% of total administered dose)					
GIT Contents						
Crop	33.32±4.37	24.27±12.45	4.46± 3.80	.42± .33	.05± .08	.12± .07
Provento.-gizz.	8.24±1.54	1.36± 1.13	.76± .37	Nil	Nil	Nil
Duod.-jejunum	4.85±1.83	2.41± 1.76	1.40± 1.37	.02± .02	Nil	Nil
Ileum	1.41± .65	3.68± 1.44	1.69± .52	.10± .11	.04± .07	.04± .07
Large Intestine	3.45± .24	5.78± 3.01	14.99± 6.32	9.10±5.15	7.56±6.00	1.95±2.37
GIT Wall						
Crop	2.50±1.50	1.31± .78	.55± .36	.13± .07	.01± .01	.01± .01
Proventriculus	.60± .43	.04± .03	.03± .03	Nil	Nil	Nil
Gizzard	1.67±1.33	.21± .15	.22± .13	Nil	Nil	Nil
Duod.-jejunum	1.38± .55	.29± .18	.17± .15	Nil	Nil	Nil
Ileum	.43± .38	.36± .19	.06± .05	Nil	Nil	Nil
Large Intestine	1.38± .71	2.14± 1.56	5.89± 5.12	1.26±1.06	.46± .18	.31± .30
Body						
Whole blood	2.31±1.44	.89± .33	.78± .35	.24± .22	.17± .12	.05± .06
Plasma	1.75± .76	.52± .15	.49± .21	.17± .16	.13± .12	.04± .03
Breast muscle	2.10± .67	.93± .62	.85± .37	.99± .69	.85± .57	.73± .57
Thigh muscle	2.31± .65	1.05± .71	1.07± .76	.94± .76	.84± .48	.85± .48
Abdominal fat	.16± .11	.13± .07	.04± .05	Nil	Nil	Nil
Heart	.07± .03	.01± .02	Nil	Nil	Nil	Nil
Liver	.74± .45	.23± .08	.13± .12	.07± .05	.06± .07	.06± .05
Bile	.25± .13	.22± .14	.20± .12	.09± .04	.06± .03	.01± .01
Kidney	1.98±1.18	.20± .02	.23± .06	.05± .03	.03± .02	.03± .03
Excreta (cumulative)	7.58±3.11	52.29±15.97	56.53±13.15	86.27±2.80	82.76±7.78	85.34±9.77
Total	78.48	98.32	90.54	99.85	93.04	89.63
Egg Yolk[2]	---	.02	.14	.08	.26	.11
Albumen[2]	---	.08	.45	.29	.65	.23

[1]All values are the average ± standard deviation of three hens each given 1.6 x 10^{6} dpm ^{3}H-DON/kg body weight.

[2]Values at 3, 6, 12, 24 and 48 h are the average from 2, 1, 1 and 3 eggs, respectively.

and ceca is normal with fowl (Moran 1982) and accounts for label distribution throughout the large intestinal complex while being comparatively low in the ileum.

Radioactivity in the wall of the GIT and in body tissues was low when disappearance from the GIT and appearance in the excreta were most extensive. Eggs laid throughout this time also had low counts with maximal values occurring almost 24 h after peak levels in the excreta. This delay and the differential between albumen and yolk relates to the chronology of events in egg assembly. Albumen is formed in total

through the preceeding 24 h whereas yolk synthesis had been initiated about 6-7 days earlier. The low tissue and egg levels of radioactivity attributable to ^{3}H-DON, and their rapid clearance agrees with the results of Prelusky et al. (1986, 1987) after dosing hens with ^{14}C-DON.

Relative radioactivity associated with the liver and bile in present experimentation involving ^{3}H-DON was noticibly less than observed by Prelusky et al (1986) where ^{14}C-DON was used. The ^{3}H was located at the C-3 whereas the ^{14}C was uniformly distributed. Loss of the ^{3}H during metabolite formation is unlikely if glucuronidation is the means of detoxification. Corley et al. (1985) injected C-3 labeled ^{3}H T-2 toxin into swine and found that the ^{3}H appeared as metabolites glucuronated at the C-3 hydroxy group. The greatest proportions of these metabolites were excreted in the urine.

Lun et al. (1988) previously showed that DON as such largely disappeared from the upper jejunum and could not be detected in portal blood nor be found to any great extent in the excreta. ^{3}H-DON used in the present study also disappeared from the upper part of the GIT. The label was not retained by any body tissues but rapidly appeared in the urine. Fowl appear to cope with ingested DON by altering the molecule shortly after absorption such that it has reduced toxicity, does not express affinity for body tissues, and can rapidly be removed from the vascular system by the kidney.

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