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Disappearance of Deoxynivalenol from Digesta Progressing along the Chicken's Gastrointestinal Tract after Intubation with Feed Containing Contaminated Corn

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Deoxynivalenol (DON) is a mycotoxin found in grain usually because of Fusarium graminearum contamination. Domestic fowl have been shown to be refractory to DON when most mammals would probably exhibit clinical signs or lesions (Trenholm et al. 1984; Hamilton et al. 1985). Gross pathology is not apparent with broiler chickens given contaminated corn until DON concentration is over twice the level with the worst of known grain contaminations (Moran et al. 1982).

Tolerance is probably related to the fowl's ability to rapidly alter DON to a less toxic form for ready excretion. Lun et al. (1986) gave laying hens feed formulated to contain 83 ppm DON which came from heavily contaminated corn. No DON could be detected in eggs and tissues while only 5% of that ingested could be recovered in the excreta as such. Prelusky et al. (1986) intubated labeled DON into chickens and found less than 1% of the label in association with the plasma 2 hr later while 78% appeared in the excreta after 24 hr.

In the present experiment, laying domestic chickens were intubated with contaminated feed and DON concentration was measured in the digesta as it progressed along the gastrointestinal tract (GIT). In vitro incubation of DON in fluids obtained from the main parts of the GIT addressed DON's possible alteration in the lumen as opposed to absorption.

MATERIALS AND METHODS

Contaminated corn was obtained by inserting Fusarium gramineraru m impregnated toothpicks into cobs at the early silk stage of development. After maturation, the

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Table 1. Composition of contaminated feed

Ingredient	(%)	Analyses	(%)
Control Corn	60.97	Moisture	12.31
Contaminated corn	8.00	Crude protein	16.44
Soybean meal	19.50	Ether extract	2.71
Cr-soybean meal	1.50	Crude fiber	2.92
Dicalcium phosphate	1.50	Calcium	3.30
Limestone	7.50	Phosphorus	.71
DL-methionine	.03	(DON ppm	22.10)
Iodized salt	.25	(Cr ppm	2.20)
Vitamin mix[1]	.50		
Mineral mix[2]	.25		
Total	100.00		

[1]Supplies the following per kg of feed: vit. A, 8000 IU; vit. D, 1600 ICU; 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, 7.5 mg; biotin, .5 mg; ethoxyquin, 125 mg.
[2]Mineral mix supplied the following in mg/kg feed: manganese, 55 as MgO; zinc 50 as ZnO; copper 5 as $CuSO_4$; iron 30 as FeO.

dried whole ear was ground and incorporating into a complete feed (Table 1). Chromium (Cr) from sodium dichromate mordanted soybean meal served as a feed marker (Uden et al. 1980).

All birds were 30 weeks of age and averaged 1.52±.06 kg. Each was housed in single wire cages and received 14 hr of light. Control feed had regular corn and soybean meal replace the contaminated and marker ingredients, respectively. Hens were given control feed 2 weeks prior to the experiment. On treatment day, 30 g of the DON-Cr feed was intubated 1.5 hr after lighting of the room. Birds were returned to their cage where they again had ad libitum access to control feed and water until their sacrifice.

Six birds were anesthetized with halothane at 0, 1.5, 3, 6, and 9 hr after intubation, and blood was taken from the portal vein and heart using heparinized syringes. Cervical dislocation followed and the crop, proventriculus-gizzard, duodenum-jejunum (above yolk stalk), jejunum (below yolk stalk) to ileum at colon, and colon with ceca and cloaca were isolated by ligation. Plasma from blood and the lumenal contents from each section of the GIT were frozen (-18°C).

Fluids from the GIT intended for in vitro incubation were obtained from hens of similar age. All birds had been receiving the same control feed and were sacri-

ficed for their GIT contents as described earlier. Fluid from the proventriculus-gizzard was obtained by pressurizing the lumenal contents in a 100 ml syringe and filtering the expressed fluid through cheesecloth. Fluid from the small intestine was the supernatant after centrifugation of entire contents (10 min, 4°C, 1700xG). Each source fluid represented a composite from several birds. Once collection was complete, 50 ml was mixed with 10 or 25 ug of DON (Myco-Lab Co, Chesterfield, MD) and incubated at 41°C in a shaker-water bath. Samples were taken at 0, 1.5, 6, 12 and 24 hr for triplicate DON analyses.

Contents from the entire large intestinal complex were obtained and continually purged with CO_2 to maintain anaerobic conditions at all times. This composite was mixed with the anaerobe culture media of King et al. (1984) as a substrate in substitution for timothy hay which had been excluded, then 100 ml of the resultant mixture was transferred to flasks having 20 and 50 ug DON. Once the DON had dispersed, portions were incubated in screw-cap tubes at 41°C that were constantly being rotated. Tubes were removed at 0, 1.5, 3, 6, 12 and 24 hr for DON analyses in triplicate.

DON analyses of feed, lyophilized GIT contents, bile, and excreta employed the extraction, derivitization and HPLC quantitation procedures described by Lun et al. (1986). DON in plasma and GIT cultures was measured on 1 and .5 ml samples, respectively, to which 4 and 4.5 ml of 90% acetonitrile was added. After mixing and centrifugation, the supernatant was placed on an alumina-charcoal column and eluted with acetonitrile:water (84:16 v/v). Eluates were evaporated, then DON in the residue was derivitized and quantitated as with the other samples.

DON measurements were statistically analyzed by weighted regression with time using a stepwise procedure (SAS, 1985). Weighting was necessary because variances were not consistently homogenous with time after intubation or incubation. Significance was arbitrarily set at $P<.10$.

RESULTS AND DISCUSSION

Recovery of the Cr marker was over 90%, regardless of time after intubation (Table 2). A similar recovery of DON only occurred immediately after intubation when most of the original dose was confined to the crop (Table 3). Thereafter, DON as such disappeared, particularly within the first 1.5 hr. Reduced recovery of DON at this time can largely be attributed to aboral movement of digesta because its relationship with Cr

Table 2. DON-Cr ratio in digesta along the hen's GI tract with time after administration of contaminated feed[1]

Time after intubation[2]	Crop	Prevent.-gizzard	Small Intestine Upper	Small Intestine Lower	Large intestine	Excreta	Recovery
	(Ratio of DON:Cr)						%
0	9.3± .90	4.2±1.44	6.6±3.58				95.3±3.28
1.5	8.2±1.30	1.3± .40	.6± .33	.2±.10	.3±.10	1.4±.61	98.0±2.33
3	7.8±1.32	1.3± .73	.9± .28	.4±.41	.3±.13	.9±.43	96.8±6.35
6	6.1±3.98	1.3± .48	.5± .33	.4±.19	.4±.10	.3±.09	96.3±8.74
9	3.9±1.48	2.5±1.67	.8± .21	.5±.22	.8±.80	.5±.33	93.1±3.97
Regression Analysis							
Intercept	9.31	---[3]	---[3]	.18	.23	2.24	---
Time	-.59	---	---	.03	.01	-.60	---
$(Time)^2$	--	---	---	---	---	.04	---
R^2 (%)	99.04	---	---	85.79	92.15	99.09	---
Sx·y	.18	---	---	.30	.22	.23	---
P<	.01	---	---	.07	.04	.09	---

[1]All values represent the mean from six birds ± standard deviation.
[2]Time after a 30 g dose of feed containing 663 ug of DON and 66 ug of chromium.
[3]Regression, P>.10.

Table 3. Recovery of DON from digesta along the hen's GIT tract with time after administration of contaminated feed[1]

Time after intubation[2]	Crop	Provent.-gizzard	Small Intestine Upper	Small Intestine Lower	Large intestine	Excreta	Total
0	90.8±12.67	3.7±3.57	.3±.07	NA	NA	NA	97.8± 9.32
1.5	44.0± 5.85	2.5±1.37	.6±.39	.4±.18	.1±.01	.1± .09	47.8± 5.14
3	28.4± 6.56	2.3±1.11	.6±.30	.9±.59	.4±.26	.7± .31	33.2± 7.25
6	16.1±10.48	1.3± .39	.3±.07	.6±.30	.4±.01	.8± .09	19.4±10.71
9	9.8± 9.36	1.3± .99	.3±.16	.2±.19	.3±.11	2.2±1.08	14.1±10.41
Regression Analysis							
Intercept	76.60	3.55	---[3]	---[3]	---[3]	-.09	84.18
Time	-20.69	-.61	---	---	---	.16	-23.02
$(Time)^2$	1.51	.04	---	---	---	---	1.75
R^2 (%)	91.41	95.86	---	---	---	94.45	91.75
Sx·y	1.20	.19	---	---	---	.97	1.35
P<	.08	.04	---	---	---	.03	.08

[1]All values represent the mean from six birds ± standard deviation.
[2]Time after 30 g dose of feed containing 663 ug of DON.
[3]Regression, P>.10.

for that portion remaining in the crop was not substantially altered. Accordingly, over 50% of the DON originally dosed should have passed into the gastric area and beyond.

Recovery of DON within the proventriculus-gizzard and all areas following was low, regardless of time after intubation. Disappearance of DON from the gastric area probably isn't due to an alteration of form or absorption even though a precipitous drop in its ratio with Cr occurred. Near complete recoveries of DON were obtained after in vitro incubation with gastric juice (Table 4). Similarly, epithelial cell structure in the proventriculus and construction of the koilin lining of the gizzard argue against absorption (Moran, 1982); however, both of these tissues do suffer from high concentrations of DON (Moran et al. 1982).

Most likely, DON and Cr separated in transit through the gastric area. Cr when mordanted on soybean meal is in particulate form while DON's amphilic character permits a partial water-solubility. Motility of the gizzard is such that fluids rapidly evacuate while particulates are retained (Moran, 1982). Under these terms, DON:Cr should have increased in the small intestine, however, the converse occurred. Alteration of DON to another form while in the lumen of the small intestine to account for this reduction in ratio again seems unlikely. Incubation of DON in juice from the small intestine did lead to a decrease in DON recovery, but meaningful change only occurred after an extended period when associated pH indicated sustantial microbial activity (e.g. 6.8, 6.3, 4.7 and 3.5 at 0, 6, 12 and 24 hr, respectively).

Enterocytes are constructed for rapid and extensive absorptive activity. While no DON was detected in the blood from the portal vein or heart (<20 ppb), trace amounts appeared in bile (ca 100 ppb). After dosing hens with labeled DON, Prelusky et al. (1986) reported that the liver and bile had high counts. Presumably there is a rapid post-absorptive modification of DON by enterocytes then hepatic removal and elimination of the altered form in bile.

Unabsorbed DON appears to be altered in the large intestine. In vitro incubation of DON in media having large intestinal contents (cecal and colonic) led to a reduction in DON concentration at a considerably greater rate than occurred with the small intestinal juice. The associated microbes are concentrated and operate anaerobically to produce volatile fatty acids by fermentation of fiber as do those in the rumen.

Table 4. Recovery of DON when incubated in vitro at high and low concentration with fluid contents from the hen's GI tract[1]

Incubation time at 40°C	Gastric Fluid		SI Fluid		LI Fluid	
	Hi DON	Lo DON	Hi DON	Lo DON	Hi DON	Lo DON
(Hr)	(ng/ml)					
0	202±11.0	497±13.1	194± 5.1	508± 3.0	184± 83	461±13.1
1.3	194± 7.1	506±14.6	196±10.0	494± 6.4	133±27.5	396±37.1
3	203±10.2	505±15.3	198± 9.6	490± 8.5	127±29.4	330±22.8
6	207± 7.0	496± 8.3	204± 8.5	494±24.2	63±12.4	240±58.5
12	197± 6.0	494± 4.6	185± 4.5	486±10.7	19± 2.4	106±44.3
24	192± 7.4	501± 8.0	178± 8.2	472±13.1	9± 1.3	78±19.1
Regression Analysis						
Intercept	---[2]	---[2]	197.13	505.06	179.92	459.70
Time	---	---	-.81	-1.59	-19.77	-44.62
Time^2	---	---	---	---	.53	1.20
R^2 (%)	---	---	65.61	70.39	99.45	99.95
Sx·y	---	---	.81	1.06	.94	.23
P<	---	---	.05	.04	.01	.01

[1]All values are based on triplicate analyses from one incubate of fluids from the proventriculus-gizzard, small intestine and large intestine.
[2]Regression P>.10.

Rumen microflora have been shown to readily reduce DON's epoxide group (King et al. 1984; Cote et al. 1986).

Present experimentation has shown that DON as such largely disappears from the GIT between the crop and jejunum. This disappearance is presumed to have occurred because of its absorption by the enterocyte and conversion to another form. High radioactivity in the liver and bile in birds given labeled DON suggests that the metabolite is being excreted in association with bile back into the small intestine.

REFERENCES

Cote, L-M, Nicoletti, J, Swanson, SP, Buck, BB (1986) Production of deepoxydeoxynivalenol (DOM-1), a metabolite of deoxynivalenol, by in vitro rumen incubation. J Agric Food Chem 34:458-460.

Hamilton, RG, Trenholm, HL, Thompson, BK, Greenhalgh, R (1985) The tolerance of white leghorn and broiler chicks and turkey poults to diets that contained deoxynivalenol (vomitoxin) - contaminated wheat. Poultry Sci 64:273-286.

King, RR, McQueen, RE, Levesque, D, Greenhalgh, R (1984) Transformation of deoxynivalenol (vomitoxin) by rumen organisms. J Agric Food Chem 52:1181-1183.

Lun, AK, Young, LG, Moran, ET, Hunter, DB, Rodrignez, JP (1986) Effects of feeding hens a high level of vomitoxin contaminated corn on performance and tissue residues. Poultry Sci 65:1095-1099.

Moran, ET (1982) Comparative Nutrition of Fowl and Swine - The Gastrointestinal Systems. Office Educational Prac, Univ Guelph, Canada.

Moran, ET, Hunter, DB, Ferket, P, Young, LG, McGirr, LG (1982) High tolerance of broilers to vomitoxin from corn infected with Fusarium graminearum. Poultry Sci 61:1828-1831.

Prelusky, DB, Hamilton, RMG, Trenholm, HL, Miller, JD (1986) Tissue distribution and excretion of radioactivity following administration of ^{14}C-labeled deoxynivalenol to white leghorn hens. Fund Appl Toxicol 7:635-645.

SAS (1985) SAS User's Guide. Statistical Analysis System Inc. Cary, North Carolina.

Trenholm, HL, Hamilton, RMG, Friend, DW, Thompson, BK, Hartin, KE (1984) Feeding trials with vomitoxin (deoxynivalenol) - contaminated wheat: effects on swine, poultry, and dairy cattle. J Am Vet Med Assoc 185:527-531.

Uden, P, Colucci, PE, VanSoest, PJ (1980) Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. J Sci Food Agric 31:625-632.

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