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Effect of Deoxynivalenol (Vomitoxin) on Fertility, Pregnancy, and Postnatal Development of Sprague-Dawley Rats

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A diet containing 20 ppm (μg/g) of purified deoxynivalenol (DON) was fed to male and female Sprague-Dawley rats for 60 and 15 days, respectively, before breeding. Rats consuming feed amended with DON throughout pregnancy and lactation showed no clinical signs of toxicity, nor did the control or pair-fed control groups. Male rats in the DON treatment group showed no feed refusal but were less efficient than males in control groups in converting feed into body mass. Feed refusal in female rats varied with stage of pregnancy. Before breeding, overall feed consumption was similar in all groups, but in the DON treatment group there was significant feed refusal for the first 2 days. Feed conversion efficiency was reduced in the DON treatment group. Pregnant and lactating rats fed DON-treated feed ate 6% less than did control rats fed solvent-treated feed. Although pair-fed control rats ate 14 to 21% less than rats in the DON treatment group, their body weights were greater than those of the DON group rats throughout most of the feeding trials, indicating that DON has a toxic effect. Only 50% of the matings between DON group rats resulted in pregnancy, compared with 80% in the control groups. There were no differences detected among groups in ratio of male to female pups, survival rate, or average litter number and weight. Pup weight gains in all groups were comparable through postnatal day 14. From day 14 to 21, however, male and female pups in the control group had significantly better weight gains than the others, presumably a result of the effect of the toxin and undernutrition, respectively, on the pups in the DON and pair-fed groups. Limited cross-fostering studies suggested that the DON effect may not be mediated through milk of the lactating dam. There were no treatment-related histologic abnormalities in testes or ovaries.

Deoxynivalenol (3,7,15-trihydroxy-12,13-epoxytrichothec-9-ene-8-one) (DON, vomitoxin) is a mycotoxin produced primarily by the fungus *Fusarium graminearum* Schwabe (perfect-stage *Gibberella zeae* (Schw.) Petch) in temperate regions of the world. Most often *Fusarium* scab in corn occurs in years that are particularly cool and wet at the time of harvest (2) and is referred to as stalk or cob rot. In a survey of 1981 corn in Illinois, levels of DON ranged from 0.1 to 41.6 ppm (μg/g), with a mean of 3.1 ppm (2). Extensive scab or head blight occurred in Canadian and U.S. wheat in 1980, 1981, and 1982. Zearalenone is often found in samples that contain DON (2, 5, 6, 10, 13).

Clinical signs associated with the *Fusarium*-infected Illinois corn harvest in 1981 were reproductive problems, feed refusal, reduced weight gain, diarrhea, emesis, and death (2). In laboratory rats, low levels of DON (5 ppm in feed) fed throughout pregnancy do not adversely affect pregnancy or cause birth defects (11). Intubation of mice on days 8 to 11 of pregnancy with higher levels of DON results in high levels of embryo lethality at doses of 5 mg and more per kg of body weight and embryo toxicity at doses of 2.5 and 5 mg/kg (8). Additional experiments (7) in which DON was fed to mice in the diet at levels as high as 2 mg/kg show dose-dependent reductions in feed and water intake and body weight of the parent generation, the number of live pups and postnatal survivors, and postnatal body weight, as well as other adverse effects.

The purpose of this investigation was to determine the effects of a dietary level of 20 ppm (μg/g) of purified DON fed throughout the experiment on fertility, pregnancy, and postnatal development of Sprague-Dawley rats. This level was chosen because it exceeds the mean DON concentration previously encountered in the field and can therefore provide toxicological data beyond that which has been reported previously (11).

MATERIALS AND METHODS

Toxin. DON was produced with *Fusarium graminearum* (NRRL 5883) cultured on corn as previously described (17). Purity of DON was estimated to be 96% as determined by gas chromatography and gas chromatography-mass spectrometry of the trimethylsilyl-ether derivatives; the remaining 4% was 3,15-dihydroxy-12,13-epoxytrichothec-9-ene-8-one. There were no detectable levels of other tricothecenes or zearalenone in the rodent feed (Charles River Certified Rat Feed; Agway, Inc., Syracuse, N.Y.). The toxin was incorporated into feed as previously described (11) with ethanol as a solvent, and the mixture was freeze-dried, broken into cubes, and stored frozen until used (12).

Experimental design. Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, Ind.), 190 to 210 g, were acclimated to the animal facility for 1 week and then randomly assigned to groups (10 rats per group). Female rats weighing at least 165 g were similarly assigned (25 per group). The control group received feed that had been treated with solvent (ethanol) before mixing and freeze-drying, the treatment group was fed a diet containing 20 ppm (wt/wt) of DON, and the third group was fed feed as controls but pair-fed to the DON treatment group. The basis for determining the amount of feed to provide to rats in the pair-fed group was the mean feed consumption of rats in the DON treatment group for the previous 24-h period. Any feed

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TABLE 1. Effect of dietary DON on feed consumption and body weight of dams[a]

Group	Feed consumption (g/rat/day) ± SD				Body wt of dams (g) ± SD			
	Males (n = 10)	Females			Prebreeding (treatment day 15)	Sperm positive	PND 1	PND 21
		Prebreeding (n = 25)	Pregnant	Lactating				
Control	24.6 ± 2.0	19.7 ± 1.8	28.0 ± 1.3	51.4 ± 3.1[b]	243.4 ± 20.1	251.3 ± 24.0[c]	302.0 ± 22.4[d]	284.5 ± 21.9
DON	27.3 ± 1.9[e] (+ 11%)	19.0 ± 2.1 (−3.5%)	26.2 ± 3.8 (−6.4%)	48.2 ± 3.7[f] (−6.2%)	234.0 ± 14.1	232.4 ± 15.4[g]	273.4 ± 18.9	279.5 ± 16.1
Pair-fed control	24.9 ± 1.2 (+ 1%)	17.6 ± 0.6[h] (−10.6%)	22.0 ± 3.5[i] (−21.4%)	44.4 ± 4.0[j] (−13.6%)	243.3 ± 14.7	247.0 ± 10.7[c,g]	276.9 ± 13.2	252.9 ± 9.6[k]

[a] Parenthetical values are percentage of change compared with the control group.
[b–k] Values with different superscripts differ significantly ($P < 0.05$) for each parameter.

remaining in the hoppers of pair-fed rats was discarded before new feed was added. Male rats were maintained in each group for 60 days, and female rats were maintained for 15 days before breeding. Two female rats were randomly assigned to one male of the same group for breeding, and sperm checks were performed the next morning. When female rats tested sperm positive, they were caged singly for the remainder of the study, continued on the assigned diet, and provided with bedding (Aspen Bedding; MSP Feeds) on day 17 of pregnancy. Each sperm-positive rat was replaced with one of the five remaining rats in a group until breeding was completed.

Rats were identified by ear notch and housed individually in suspended wire cages in racks equipped with automatic watering systems and automatic flushing. Pregnant rats were transferred to racks equipped with inserts to prevent bedding material from falling out. Automatic flush was turned off on these racks, and bedding was changed as it became soiled. Temperature was maintained at 23 ± 3°C, and relative humidity was maintained at 50 to 70%. Fluorescent lighting was turned on at 6:00 a.m. and off at 6:00 p.m. to provide a 12-h light-dark cycle. Feed and water were available ad libitum to all groups during breeding. Feed consumption was recorded daily, except when males and females were breeding, and during delivery of pups.

Rats were weighed weekly and observed twice daily for any unusual signs. Before the breeding period described above, male rats were tested for ability to sire a litter. Dams delivering pups were frequently observed to reduce the likelihood that cannibalism would go unnoticed. Postnatal day 1 (PND 1) was considered the first morning after all pups were delivered and cleaned. Pups were sexed, weighed, and examined for defects at this time. On PND 4, litters were culled to 10 with equal numbers of males and females, if possible. Pups were weighed on PNDs 1, 4, 7, 14, and 21. Limited cross-fostering was achieved on PND 1 by switching the pups between dams in the DON group and either control group. Groups of pups were numbered as follows: 1, control; 2, pair-fed control; 3, 20 ppm of DON; 4, DON dam cross-foster control pups; 5, control dam cross-foster DON pups; 6, pair-fed control cross-foster DON pups; 7, DON cross-foster pair-fed control pups. Dams continued to consume the assigned diet.

Testes and ovaries were preserved in Bouin fixative, dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and p-aminosalicylic acid. All sections were evaluated for histologic abnormalities. One hundred tubule cross sections from each rat were examined and scored as (i) having mature spermatids aligned at the tubule lumen, (ii) being nonfunctional with little evidence of active spermatogenesis, or (iii) other (1).

Statistical analysis. Continuous variable data were analyzed for statistical differences ($P < 0.05$) by the Bartlett test for homogeneity of variance and then subjected to analysis of variance or the appropriate t test (14). The litter was considered the sample unit, where appropriate. Nonparametric data were analyzed by the Kruskall-Wallis test. Categorical data were analyzed by the chi-square test with Yates correction.

RESULTS

The animals in the three groups showed no unusual behavior or clinical signs of toxicity. Feed refusal varied with sex. Male rats in the DON treatment group consumed somewhat more feed than did male rats in control groups (Table 1). The conversion of feed into body mass by rats eating feed amended with DON was less efficient than in control groups. At the start of the experiment rats in all three groups weighed 200 to 202 g; 60 days later, when breeding began, rats in the DON treatment group weighed 289 g, compared with 294 g in each control group. Because the group eating feed with added DON consumed more but weighed less, the conversion efficiency must have been decreased. Among females in the DON treatment group, a decrease in feed consumption was detected that varied with stage of pregnancy. Females eating DON-containing feed before breeding consumed about the same amount of feed as did the controls. However, on the first day of treatment they consumed only 47% as much feed as controls and on the second day, 70%. Pair-fed controls ate 94% as much feed as controls on the first day. The DON group eventually consumed feed at a rate comparable to that of the control group. This was indicated by only a 3.5% reduction in feed consumption over this 2-week period compared with an almost 11% reduction in the pair-fed group. As in the males, feed conversion efficiency was reduced in the DON treatment group (−0.6% compared with +2.7% in the control group). A decrease in weight of 2 g over a 14-day period (Table 1) coincided with the relatively minor reduction in feed consumption. Pair-fed control animals gained 4 g during this period while eating substantially less feed.

Pregnant DON group rats ate about 6% less feed than did controls, but this was not a statistically significant difference. Pair-fed rats ate 21% less feed during the same period. Both DON group rats and pair-fed controls consumed significantly ($P < 0.05$) less feed compared with controls during lactation. Female rats consumed DON-containing feed at a rate 6% lower than controls, and pair-fed controls ate significantly less (14% less than the control group). In each group, sperm-positive females that were not pregnant ate approximately the same amount of feed.

Even though the pair-fed rats consumed less feed than the

TABLE 2. Effect of dietary DON on female Sprague-Dawley rats and litter size and viability

Group	Females			Pups		
	No. sperm positive	No. pregnant	% Pregnant	Mean no. per litter		Viability index ± SD
				Male	Female	
Control	20	16	80	6.1	4.9	97.8 ± 4.3
DON	22	11	50[a]	5.5	5.6	93.9 ± 11.2
Pair-fed control	15	12	80	6.5	7.0	99.4 ± 1.9

[a] Significantly lower compared with combined total of control rats (control plus pair-fed control).

rats receiving 20 ppm of DON in their diet during pregnancy, the pair-fed controls weighed more on the day of delivery (Table 1). At the end of the lactation period, pair-fed control rats weighed less than rats in the DON group.

Gestation index (the percentage of matings resulting in birth of live pups) was lower for females receiving DON than for either control group (Table 2). There was no difference among groups in ratio of males to females, viability index (the percentage of animals born that survived 4 days or longer) (Table 2), and average litter weight of males and females, although females in the DON treatment group tended to weigh less than controls from PNDs 14 to 21 (Table 3). The lactation index (the percentage of animals alive at 4 days that survived the 21-day lactation period) was 100% for the DON group and slightly lower for the other two groups. Pair-fed control pups of both sexes tended to weigh less than pups in the other two groups, but there was an average of more than two additional pups per litter in this group. Weight gains of pups through PND 14 were comparable in all groups. From PNDs 14 to 21, however, male and female pups in the control group had gains that were significantly better than those of pups in both the DON and pair-fed control groups.

Because of poor fertility in the DON treatment group, cross-fostering studies were limited to only six rats. Weight gains of group 4 (DON group dam with control group pups) exceeded those of group 1 (Fig. 1), and groups 5, 6, and 7 showed comparable gains that were smaller than those of other groups.

Histologic examination of testes and ovaries revealed no treatment-related abnormalities. The number of testicular cross sections containing mature spermatids and those showing little evidence of spermatogenesis were not significantly different among groups.

TABLE 3. Effect of dietary DON on pup weight

Group	Mean wt (g) ± SD		Wt gain (g) ± SD	
	PND 1	PND 21	PND 7 to 14	PND 14 to 21
Control				
Male	6.4 ± 0.3	51.2 ± 3.3	15.2 ± 1.3	20.8 ± 2.0[a]
Female	6.2 ± 0.4	49.3 ± 4.1	14.7 ± 1.8	19.4 ± 2.0[b]
DON				
Male	6.5 ± 0.5	47.4 ± 6.2	15.5 ± 3.1	16.4 ± 3.0[c]
Female	6.2 ± 0.6	45.9 ± 7.8	14.9 ± 3.5	15.4 ± 2.7[d]
Pair-fed control				
Male	5.9 ± 0.6[e]	42.3 ± 3.5[f]	14.3 ± 2.0	14.3 ± 1.6[g]
Female	5.5 ± 0.6[h]	39.2 ± 4.3[i]	13.5 ± 1.8	13.0 ± 1.4[j]

[a-j] Values with different superscripts differ significantly ($P < 0.05$) for each parameter. Data for males and females were analyzed separately.

DISCUSSION

There were no significant differences among groups in this study with respect to postnatal pup survival, number of pups born, sex ratio, mean pup weight, viability, or lactation index. In contrast, severe pup mortality occurred between PNDs 1 and 7 among offspring of mice receiving daily doses of 2 mg of DON per kg (7), equivalent to the 20 ppm of DON consumed by rats in the present study. We observed a decrease in weight gain between PNDs 14 and 21 that appears similar to decreased weight gains by mice eating 1.5 mg of DON per kg daily (7) during an identical period, and among survivors of the 2.0 mg of DON per kg per day treatment group.

Female rats with 20 ppm of DON in the diet had a lower feed conversion ratio than did control rats during the prebreeding period. During pregnancy the DON treatment and

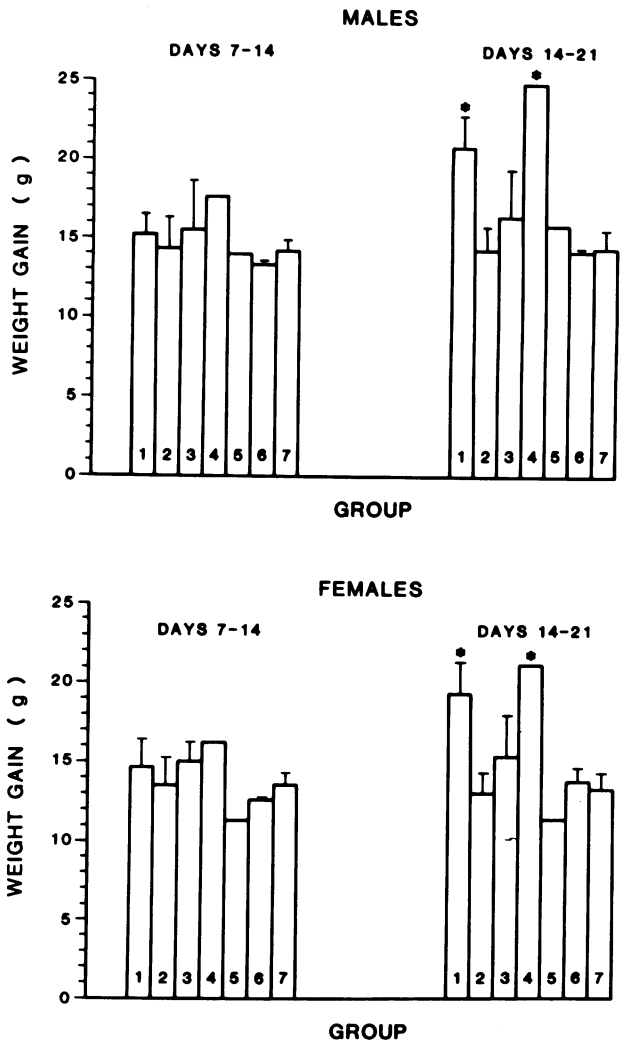

FIG. 1. Bar graphs of mean weight gains (± standard deviation) of male and female pups from PNDs 7 to 14 and 14 to 21. Groups: 1, control; 2, pair-fed control; 3, 20 ppm of DON; 4, control pups cross-fostered by DON dam; 5, DON pups cross-fostered by control dam; 6, DON pups cross-fostered by pair-fed control dam; 7, pair-fed control pups cross-fostered by DON dam. *, Statistical significance ($P < 0.05$).

pair-fed control group were comparable in weight even though the pair-fed control group ate 15% less feed. This is indicative of a toxic effect in addition to decreased weight gain caused by decreased feed consumption.

Swine are generally most sensitive to DON-contaminated feed and exhibit both emesis and feed refusal. Although not so sensitive to refusal effects as swine, rats have been shown to be responsive to DON at levels approximating those that affect swine (16). There is a dose-response effect of DON on feed intake and weight gain (4). Rats showed responses similar to those of pigs in that they lost weight when started on DON diets, and after a few days intake and weight gains increased to control levels. Feed refusal and decreased weight gains were observed in pigs fed diets containing 0.3 and 0.7 ppm of DON (15). Analysis of suspected field cases of DON-contaminated feed did not reveal high DON levels. Feed refusal was much greater for naturally infected corn samples than for feeds with equal concentrations of pure compound added (3), indicating the involvement of an additional factor in swine refusal response. Emphasis should be placed on potential effects of DON on reproduction, not on absolute levels of the toxin, because much lower levels may produce the same effects in field situations.

In the present study the fertility of Sprague-Dawley rats eating 20 ppm of DON was lower than in control groups. Conversely, other investigators found no effect on fertility of the same strain of rats after a 6-week exposure to DON (7). One possible explanation for this observed difference is that a 60- to 70-day minimum exposure of male rats to a toxin is required to allow the toxin to affect all stages of spermatogenesis (1). This difference in duration of exposure could account for the differences in the male reproductive function, but the female exposure was adequate because estrous cycle is shorter than the male spermatogenesis cycle. Because a relatively small number of rats was used in this investigation, additional study is indicated to determine whether this decrease in fertility is significant. We found no effect of DON on testis or ovary structure, but there are numerous other parameters (1) that could be affected and that would interfere with normal reproductive processes.

Pup weight gains in all groups were comparable through PND 14 of development, at which time the control group rats gained weight faster than rats in the other two groups. Pair-fed control rats gained less than those eating DON, probably the result of continued depression of feed intake and return of maternal body weight to the prebreeding weight range. In limited cross-fostering experiments, the only pups to gain at a rate comparable to controls were the control group pups nursed by a DON group dam, suggesting that the effect of DON may not be through the milk. In another study (7), mouse pups born of control dams and nursed by DON-treated dams were not significantly different in survival and postnatal weight gain from control pups reared by control dams. In the present study, DON pups nursed by either controls or pair-fed controls gained no better than pair-fed controls or those reared by their natural mothers. This suggests the effect of DON is prenatal in rats. Pair-fed control pups nursed by dams fed DON gained weight no better than did pups in the other two groups, indicating that feed intake is also an important factor. In mice (7), pups born to treated dams but nursed by control dams had improved postnatal survival and body weight gains compared with pups born to and nursed by DON-treated dams. This suggests that postnatal effects of DON in mice may also be important. Additional study of the pre- and postnatal effects of DON is required to determine the period of greatest sensitivity to DON. There are possibly species differences in response to DON between mice and rats. The best species model for farm animal and human health effects remains to be determined.

In the present study a small decrease in feed intake in conjunction with toxin resulted in decreased weight gains during pregnancy, but pup weights were normal. Not until later in the lactation period were weight gains of pups adversely affected. More study is required to determine effects on fertility and to ascertain the developmental period of greatest sensitivity to the effects of DON. Feed conversion was adversely affected by DON in the present study, whereas feed refusal was minimal. The same effects in farm animals would result in adverse economic effects, the cause of which could go unrecognized in species that do not vomit or refuse feed in response to the presence of DON. If animals become weakened by exposure to DON, secondary effects may occur.

The consequences of multitoxin exposure must be considered because rarely is only one toxin present in a particular food source. Although DON often occurs with zearalenone, the effects on animals of combined exposure have received only limited study (9). Small amounts of zearalenone and DON in combination could cause abnormalities such as infertility and decreased weight gains (feed conversions), which result in currently unrecognized animal losses.

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