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Optimization of the Mouse Bioassay for Deoxynivalenol as an Alternative to Large Animal Studies

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Toxicological experiments involving large animals are more difficult to conduct than those using small animals for several reasons, especially considering the numbers of animals required per treatment to satisfy statistical considerations. Not only are there problems in managing and handling the animals, but it can be extremely difficult and costly to obtain the required amounts of the toxin/metabolite. Mice and rats are often used because they are readily available at low cost and in large numbers, and they require less space and much smaller quantities of the test material.

Recent studies at the Centre for Food and Animal Research (CFAR) have shown that certain combinations of deoxynivalenol (DON, vomitoxin) with *Fusarium graminearum* metabolites (Rotter *et al.*, 1992b) and T-2 toxin (Friend *et al.*, 1992) can lead to interactions in their effects on swine. The mouse bioassay as a model for the pig in mycotoxin studies is intended only for short-term experiments, to screen the metabolites and their potential interactions (Rotter *et al.*, 1992a).

Although information is available on the toxic effects of dietary DON in mice (Arnold *et al.*, 1986; Forsell *et al.*, 1986, Tryphonas *et al.*, 1986; Hunder *et al.*, 1991), the impact of factors such as dietary composition and gender (Iverson *et al.*, 1985) on their response to DON has not been extensively reported. The present research was conducted to compare the response of male and female mice to pure dietary DON and then to compare the response of male mice to diets with high and low fat content.

MATERIALS AND METHODS

Weanling 3-wk-old outbred male and female mice (ICR, weighing 16-18g) were obtained from the CFAR Research Farm and housed singly in transparent polypropylene cages. Each cage was equipped with a stainless-steel wire lid,

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individual feeder and a layer of heat-treated hardwood shavings. Water was provided *ad libitum* to each cage through an automatic watering system. The mice were kept in an environmentally controlled room (22 ± 2°C) on a 12-h light/dark cycle and given experimental diets for a period of 2 weeks. Two mash-mouse chow diets (Purina Mills, St. Louis, Mo) were fed *ad libitum*, one a high-fat diet (5015) in both experiments and the second a low fat diet (5001) in the second experiment only.

In Experiment 1, 40 male and 40 female mice were first assigned to 10 blocks of specific weight ranges for each gender and then within each block randomly assigned to 4 dietary treatments (0, 2, 4, 8 mg DON/kg diet). In Experiment 2, 80 males were assigned to 10 blocks according to body weight and then within each block randomly assigned to 8 dietary treatments: 4 levels of DON (0, 3, 6, 9 mg DON/kg diet) each at 2 energy levels (low-fat, high-fat). The specified amount of purified DON (99.1%) was mixed into the diet (Rotter *et al.* 1992a). Sufficient quantities of diet were prepared for the duration of the experiment and stored at 4°C. At the end of Experiment 1, blood samples were collected for hematological analysis [red blood cell (RBC), hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration] and analyzed using automated procedures according to manufacturer's recommendations (Sysmex 800, TOA Medical Electronic Corporation, Kobe, Japan).

Both mouse chows were tested for DON (Trenholm *et al.*, 1985) and other trichothecenes (Croteau *et al.*, 1994) prior to use and found to contain 0.06 mg DON/kg diet. The final diets were analyzed for DON (Trenholm *et al.*, 1985) to confirm dietary concentrations and to ensure a uniform mix. Analyses of variance were applied to the weight gain, food consumption and food efficiency data for each week of the two experiments (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

Ingestion of DON by the mice resulted in an immediate reduction in food intake and growth during the first 7 days as indicated in Table 1. A significant ($P < 0.05$) DON x sex interaction suggested that the degree of growth depression attributable to DON differed with sex, i.e., it was more pronounced in males than in females. The males seemed to be considerably more sensitive to dietary DON than the females. As the level of DON increased from 0 to 8 mg/kg diet, the weight gain of the males during the first week was 0.42 g/d lower than weight gain for the controls, while weight gain for the high dose females was 0.12 g/d less compared to controls. The dose intake was lower for the males (1.49 mg DON/kg b.w./d) than it was for the females (1.59 mg DON/kg b.w./d). The males were also much more efficient in utilizing food as reflected in the food conversion values, and the interaction was again significant ($P < 0.05$). During the second week, the numerical decrease in food intake was much greater in the males than in the females.

Iverson *et al.* (1985) also reported that males were more sensitive to DON than females, and in addition, that DON was more toxic to mice than rats. Recently, Green *et al.* (1993) noted that hematuria was detectable in male mice (B6C3F1) fed 2 ppm of DON after 4 weeks of exposure. A similar condition in females was observed only after 8 weeks of exposure to 10 and 25 ppm of DON (in AIN76A) mice. However, due to differences in diet and strains of mice, those results are not directly comparable with those of the current study. Hematological analysis indicated sex differences for various parameters (data not shown) and a DON effect (P = 0.048) for RBC count for both sexes. The RBC values ($10^6/\mu l$) were 9.23, 8.30, 9.19, 9.11 for males and 10.56, 9.30, 9.79 and 9.79 for females fed the 0, 2, 4 and 8 mg DON/kg diets, respectively. In the current study, it was interesting to observe some adaptation to dietary DON for the males and compensatory growth for the females during the second week. In comparison with mice, pigs show similar growth patterns when fed DON-contaminated diets (Rotter *et al.* 1992a). Depending upon

Table 1. Weight gain (WG), food intake (FI) and food conversion (FC) of male and female mice fed control and DON-containing diets over a 14 day period (Expt. 1).

DON dose[1] mg/kg diet		WG (g/d)		FI (g/d)		FC[2]	
		0-7	7-14	0-7	7-14	0-7	7-14
Male							
0	(0.00)[3]	1.39	0.33	4.47	4.99	0.312	0.066
2	(0.37)	1.20	0.33	4.14	4.81	0.289	0.069
4	(0.76)	1.09	0.36	4.22	4.77	0.259	0.072
8	(1.49)	0.97	0.27	4.03	4.57	0.243	0.059
Female							
0	(0.00)[3]	0.87	0.28	4.08	4.84	0.218	0.059
2	(0.41)	0.86	0.28	4.01	4.90	0.216	0.055
4	(0.81)	0.89	0.34	3.98	4.90	0.225	0.069
8	(1.59)	0.75	0.39	3.82	4.58	0.198	0.084
SEM		0.04	0.05	0.10	0.13	0.009	0.009
ANOVA				Mean squares			
DON		0.253***	0.010	0.411*	0.778*	0.671**	0.050
SEX		2.065***	0.000	1.200**	0.131	7.825***	0.001
DON x SEX		0.107*	0.031	0.058	0.139	0.416*	0.147
Error		0.019	0.021	0.106	0.206	0.089	0.092

* P <0.05, ** P<0.01, ***P <0.001.

[1] Analyzed dietary concentrations were 0.0, 1.9, 3.8 and 7.8 mg DON/kg diet.

[2] Weight gain/food consumed.

[3] mg DON/kg b.w./d.

the level and source of contamination, there is an initial reduction in feed consumption during the first week followed by growth depression (Friend *et al.* 1986). During the subsequent weeks, the animals are able to go through a partial or complete recovery. However, as observed with mice, male pigs are more sensitive to the effects of DON than females. Coté *et al.* (1985) reported that barrows (castrated males) had lower and more erratic weight gains than gilts after consuming DON-contaminated diets for 4 wks. Their growth rate did not return to normal during the 5th wk on a control diet.

Since the first experiment indicated that males are more sensitive than females to DON, only males were used in the second experiment to examine the influence of the two different diets (Table 2).

Table 2. Weight gain (WG), food intake (FI) and food conversion (FC) of male mice fed control and DON-containing diets (high vs low fat content) over a 14 day period (Expt. 2)

DON dose[1] mg/kg diet		WG (g/d)		FI (g/d)		FC[2]	
		0-7	7-14	0-7	7-14	0-7	7-14[3]
Factor							
0	(0.00)[4]	1.36	0.44	4.74	5.30	0.288	0.082
3	(0.60)	1.21	0.43	4.52	5.16	0.269	0.084
6	(1.23)	1.07	0.41	4.54	5.10	0.238	0.082
9	(1.77)	0.94	0.42	4.10	4.88	0.231	0.088
SEM		0.03	0.03	0.08	0.07	0.006	0.006
Diet type							
High fat (0.83)[4]		1.13	0.47	4.12	4.63	0.275	0.102
Low fat (0.98)		1.15	0.37	4.83	5.60	0.238	0.066
SEM		0.02	0.02	0.05	0.05	0.004	0.004
ANOVA				Mean squares			
Diet type (DT)		0.007	0.196*	9.693***	18.082***	2.636***	2.527***
DON		0.601***	0.003	1.375***	0.604**	1.333***	0.018
DT x DON		0.015	0.034	0.045	0.211	0.035	0.108
Error		0.018	0.018	0.119	0.097	0.079	0.068

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[1] Analyzed dietary concentrations were 0.0, 3.0, 5.8 and 8.8 mg DON/kg diet (high fat) and 0.0, 2.8, 5.7 and 8.7 mg DON/kg diet (low fat), respectively.

[2] Weight gain/food consumed.

[3] Difference in slopes between the two diets approached significance at $P = 0.077$. The food conversion values for high and low fat diets were: 0.089 and 0.075 (0 mg DON), 0.106 and 0.062 (3 mg DON), 0.102 and 0.061 (6 mg DON) and 0.111 and 0.066 (9 mg DON), respectively.

[4] mg DON/kg b.w./d.

Although both dietary formulations were based on natural ingredients, there were important differences in chemical composition between the two diets. The total digestible nutrient (%), protein (%) and crude fibre (%) contents were 76, 23.4 and 5.8 respectively, for the low fat diet and 88.0, 17.5 and 2.5, respectively, for the high fat diet. The effects of diet type and DON on food consumption were observed during both weeks. Mice consumed larger quantities of the lower fat diet (higher dose of DON) and were less efficient in utilizing the food ($P<0.001$). It is interesting to note that there was a difference ($P=0.077$) in slopes for food conversion between the two diet types during the second week. For the higher energy diet, as the DON concentration increased, the food conversion increased. For the lower energy diet, as the dietary DON concentration increased, food utilization was essentially the same over the range of DON levels, but the efficiency was less than that seen in the controls (Table 2, footnote 2).

In view of the importance of the mouse as a laboratory test animal, it is surprising that more is not reported in the literature concerning issues pertaining to experimental protocol in mycotoxin studies.

Two factors were considered in trying to improve the sensitivity of the mouse bioassay as a model system for large animals (pig) in mycotoxin studies. It was found that male mice are more sensitive to dietary DON than are female mice. In addition, the feed intake of a low energy diet was considerably higher than that of a high energy diet, which leads to a higher consumption of DON. These two factors should be considered in future experiments when screening for *Fusarium* metabolites and relating their toxicity to DON.

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REFERENCES

Arnold DL, McGuire PF, Nera EA, Karpinski KF, Bickis HG, Zawidzka ZZ, Fernie S, Vesonder FR (1986) The toxicity of orally administered deoxynivalenol (vomitoxin) in rats and mice. Food Chem Toxicol 24:935-941

Coté LM, Beasley VR, Bratich PM, Swanson SP, Shivaprasad HL, Buck WB (1985) Sex related reduced weight gains in growing swine fed diets containing deoxynivalenol. J Anim Sci 61:942-950

Croteau SM, Prelusky DB, Trenholm HL (1994) Analysis of trichothecene mycotoxins by gas chromatography with electron capture detection. J Agr Food Chem (in press)

Forsell JH, Witt MF, Tai JH, Jensen R, Pestka JJ (1986) Effects of 8-week exposure of the B6C3F1 mouse to dietary deoxynivalenol (vomitoxin) and zearalenone. Food Chem Toxicol 24:213-219

Friend DW, Trenholm HL, Thompson BK, Fiser PS, Hartin KE (1986) Effect of feeding diets containing deoxynivalenol (vomitoxin) - contaminated wheat or corn on the feed consumption, weight gain, organ weight and sexual development of male and female pigs. Can J Anim Sci 66:765-775

Friend DW, Thompson BK, Trenholm HL, Hartin KE, Boermans HJ, Panich PL (1992) Toxicity of T-2 toxin and its interaction with DON when fed to young pigs. Can J Anim Sci 72:703-711

Greene D, Azcona-Olivera J, Warner R, Pestka JJ (1993) Male predilection for vomitoxin-induced IgA nephropathy in the B6C3F1 mouse. Toxicologist 13:324 (abstr.)

Hunder, G, Schümann K, Strugala G, Gropp J, Fichtl B, Forth W (1991) Influence of subchronic exposure to low dietary deoxynivalenol, a trichothecene mycotoxin, on intestinal absorption of nutrients in mice. Food Chem Toxicol 29:809-814

Iverson F, Lok E, Nera EA (1985) Pathological and biochemical effects of vomitoxin in rodents. Toxicologist 5:6 (abstr.)

Rotter BA, Rotter RG, Thompson BK, Trenholm HL (1992a) Investigations in the use of mice exposed to mycotoxins as a model for growing pigs. J Toxicol Environ Hlth 37:329-339

Rotter RG, Thompson BK, Trenholm HL, Prelusky DB, Hartin KE, Miller JD (1992b) A preliminary examination of potential interactions between deoxynivalenol (DON) and other selected Fusarium metabolites in growing pigs. Can J Anim Sci 72:107-116

Snedecor GW, Cochran WG (1980) Statistical Methods 7th ed Ames Iowa State University Press.

Trenholm HL, Warner RM, Prelusky DB (1985) Assessment of extraction procedures in the analysis of naturally contaminated grain products for deoxynivalenol (vomitoxin). J Assoc Off Anal Chem 68:645-649

Tryphonas H, Iverson F, Ying S, Nera EA, McGuire PF, O'Grady L, Clayson DB, Scott PM (1986) Effects of deoxynivalenol (vomitoxin) on the humoral and cellular immunity of mice. Toxicol Lett 30:137-150