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Hematologic and Immunologic Toxicity of Deoxynivalenol (DON)-Contaminated Diets to Growing Chickens

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Deoxynivalenol (3a, 7a, 15-trihydroxy-12,13-epoxytrichothec-9-en-one; (DON, vomitoxin)), is a mycotoxin which can be produced in various commodities by Fusarium species of fungi. It has worldwide distribution (Vesonder et al. 1978; Mirocha et al. 1979; Pathre and Mirocha 1979; Trenholm et al. 1983; Cote et al. 1984; Hagler et al. 1984) and can cause feed refusal and emesis in swine. To date, poultry have been considered relatively insensitive to DON (Hulan and Proudfoot 1982; Moran et al. 1982; Trenholm et al. 1984; Hamilton et al. 1985). Recently, Huff et al. (1986) and Kubena and Harvey (1988) reported anemia in broiler and Leghorn chicks and reduced weight gain in broilers, and Harvey et al. (1988) reported decreased immune function in broiler and Leghorn chicks that were fed DON-contaminated wheat diets. Tryphonas et al. (1984) and Robbana-Barnet et al. (1988) noted that immune responses in mice were suppressed by DON treatments. Because DON induces changes in the hematopoietic system of chicks and alters immune response in mice and chicks, the objectives of the present study were: 1) to characterize the effects of DON-contaminated wheat diets on hematologic measurements, cell-mediated immune responses, and humoral immune responses of Leghorn chickens and 2) to measure the effects of diets formulated with purified DON on hematologic measurements and cell-mediated immune responses of broiler and Leghorn chicks.

MATERIALS AND METHODS

In the first study, sixty, one-day-old female white Leghorn chicks (Hy-Line W36) were individually weighed, randomly assigned into each of two treatment groups (3 replicates of 10 chicks per treatment) and placed in electrically heated battery brooders. One group received a control (noncontaminated) wheat starter-grower diet and the other group received a starter-grower diet formulated with wheat naturally contaminated with DON. Diets contained or exceeded the levels of critical nutrients recommended by the National Research Council (1984) and consisted of 68.5% wheat, 28.5% soybean meal-based

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concentrate (including vitamins and minerals), and 3.0% corn oil. The contaminated and noncontaminated wheat were analyzed for DON and were found to contain 26 mg DON/kg and 0 mg DON/kg, respectively. Zearalenone, T-2 toxin, ochratoxin, or aflatoxin were not detected (detection limits of 20 ug/kg). Diets were calculated to contain 0 and 18 mg DON/kg and were fed to chickens ad libitum from one day to 18 weeks of age. At 14 weeks of age, chickens were vaccinated with a oil-adjuvated killed virus Newcastle disease (ND) vaccine (Agri-Bio Corp., Gainsville, GA.). At 18 weeks of age, blood samples were drawn from each chicken and hemagglutination-inhibition (HI) titer tests for ND were conducted on the serum. Body weight gain and feed efficiency were monitored throughout the 18 week period.

In a second study, sixty male and sixty female day-old Leghorn chicks (Hyline W-36) were individually weighed, identified by numbered wingbands and assigned by sex to treatments. Four dietary treatments (3 replicates of 10 each) were formulated with the uncontaminated and DON-contaminated wheat utilized in study 1 and consisted of 1) 0 mg DON/kg, males; 2) 0 mg DON/kg, females; 3) 18 mg DON/kg, males; and 4) 18 mg DON/kg, females. Diets were fed from day 1 to 9 weeks of age. At 3 and 6 weeks of age, chicks were vaccinated with a killed ND vaccine (Agri-Bio Corp., Gainsville, GA.) and at 3, 6, and 9 weeks of age blood samples were drawn from 15 chicks per treatment for hematologic measurements and serum HI titers to ND. Hematologic measurements included RBC counts, packed cell volume (PCV), hemoglobin, mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC). Procedures for RBC, PCV, hemoglobin, and MCV were performed using a Coulter counter according to the manufacturers recommendations (Coulter Model ZM Counter with mean corpuscular volume/hematocrit computer and Model C256 size analyzer, Coulter Electronics, Hileah, FL). Hemoglobin was measured as cyanmethemoglobin. Mean cell hemoglobin and MCHC were calculated. At 3, 6, and 9 weeks of age, three chicks per treatment were euthanized and spleens aseptically removed, splenocytes harvested and stimulated with phytohemagglutinin-P (PHA--Wellcome Diagnostic Lab, Research Triangle, N.C.). Following a modification of previously reported procedures (Lee 1978), cells were stimulated by 25 ug PHA/ml, incubated for 72 hours, radioactivity counted in a liquid scintillation counter, and blastogenic responses to PHA were measured. Stimulation indices for splenocytes were calculated as follows: radioactive counts in PHA stimulated cells minus counts in unstimulated cells divided by counts in unstimulated cells.

In the third study, twenty male and twenty female one-day-old broiler chicks (Hubbard X Hubbard) were individually weighed, identified by numbered wingbands, assigned by sex to treatments, and placed into electrically heated batteries with continuous fluorescent lighting. Four dietary treatments consisted of 1) 0 mg DON/kg, males; 2) 0 mg DON/kg, females; 3) 50 mg DON/kg, males; and 34) 50 mg DON/kg, females. There were two replicates of 5 broilers/dietary treatment. Corn/soybean meal-based

starter diets contained or exceeded levels of recommended critical nutrients (National Research Council 1984) and were fed to broilers ad libitum to 3 weeks of age. Purified DON (Diversified Research Laboratories, Ltd., Toronto, Canada) was added to diets to achieve proper dosages. At 3 weeks of age, chicks were killed by cervical dislocation and spleens aseptically removed from 3 randomly selected chicks/treatment. Splenocytes were harvested, stimulated with PHA and stimulation indices calculated as described earlier.

In a fourth study, an identical design as in study 3 was utilized, excepting that Leghorn chicks (Hyline W-36) were substituted for broiler chicks and spleens from five chicks/treatment were harvested and stimulated with PHA.

Data for all response variables were subjected to analysis of variance (Snedecor and Cochran 1967) using the general linear model procedure in the SAS software (SAS 1982). Variable means for treatments showing significant differences in the analysis of variance were compared and differences were indicated using Duncan's multiple range procedure (Duncan 1955).

RESULTS AND DISCUSSION

Weight gain or feed efficiency of DON treatments did not differ from controls in any of the Leghorn or broiler studies. In study 1, the ND humoral titers of 18 week old pullets fed the DON-contaminated diets were significantly ($P<.05$) reduced (50.8 $\pm$ 40) when compared to titers of pullets (80.6 $\pm$ 60) fed control wheat diets.

In study 2, hematologic values of male chicks affected by the diets formulated with DON-contaminated wheat (18 mg DON/kg feed) included increased, then decreased MCH and MCHC for weeks 3 and 6, respectively, and decreased RBC, PCV, and hemoglobin for week 9 (Table 1). In pullets, DON-contaminated diets induced increased MCH and MCHC on week 6 and increased MCV and decreased MCHC on week 9. When compared by treatment, the stimulation index of splenocytes from female Leghorns fed DON was reduced from control females at 3, 6, and 9 weeks (Table 2). Although treatment-related differences for ND vaccination titers were not noticed (data not shown), sex-related differences did occur. On week 9, mean titers of control females were significantly ($P<.05$) increased (1369 $\pm$ 270) when compared to titers of control males (747 $\pm$ 111). Likewise, lymphoblastogenic responses (weeks 3, 6, and 9) of control pullets were higher than those of control cockerels (Table 2). The biological importance of these sex differences is unknown; however, it possibly is a natural phenomenom and necessary for females to respond rapidly and quantitatively to antigens for transmission of high levels of maternal antibodies to the egg. Conversely, the hematologic values of cockerels (Table 1) were affected to a greater degree by DON treatments and occurred earlier than those of pullets. Erythrocytes, hemoglobin, MCH, MCHC, and PCV were affected in male chicks whereas only MCV and MCHC were changed

Table 1. Hematologic values and ND HI-vaccination titers analyzed by sex and age of Leghorn chicks fed deoxynivalenol (DON)-contaminated wheat diets for 9 weeks.

Age	Treatment		RBC ($X10^6/ul$)	MCV (μm^3)	PCV (%)	Hemoglobin (g/dl)	MCHC (%)	MCH (pg)
3 wk	Male	Control	2.49 ± .08[a]	129.0 ± .71[a]	32.2 ± 1.06[a]	8.13 ± .24[a]	25.35 ± .42[b]	32.76 ± .50[b]
		DON	2.37 ± .07[a]	127.5 ± .74[a]	30.7 ± .86[a]	8.29 ± .13[a]	27.20 ± .73[a]	35.22 ± .75[a]
	Female	Control	2.71 ± .06[a]	125.5 ± .76[a]	33.8 ± .73[a]	8.45 ± .14[a]	25.06 ± .45[a]	31.28 ± .49[a]
		DON	2.53 ± .06[a]	125.5 ± .57[a]	31.8 ± .81[a]	8.15 ± .10[a]	25.79 ± .62[a]	32.40 ± .76[a]
6 wk	Male	Control	2.48 ± .05[a]	134.3 ± 1.07[a]	33.1 ± .52[a]	8.77 ± .12[a]	26.52 ± .29[a]	35.47 ± .40[a]
		DON	2.67 ± .10[a]	131.9 ± 1.06[a]	35.4 ± 1.36[a]	8.46 ± .21[a]	24.10 ± .54[b]	31.94 ± .76[b]
	Female	Control	2.62 ± .10[a]	127.7 ± .81[a]	33.0 ± .98[a]	8.13 ± .17[a]	24.79 ± .50[b]	31.48 ± .84[b]
		DON	2.39 ± .06[a]	127.6 ± .83[a]	30.4 ± .78[a]	8.38 ± .16[a]	27.68 ± .38[a]	35.24 ± .48[a]
9 wk	Male	Control	2.85 ± .05[a]	133.3 ± 1.00[a]	37.9 ± .58[a]	9.32 ± .19[a]	24.62 ± .38[a]	32.74 ± .53[a]
		DON	2.61 ± .04[b]	132.2 ± .57[a]	34.5 ± .61[b]	8.72 ± .13[b]	25.63 ± .33[a]	33.44 ± .44[a]
	Female	Control	2.65 ± .05[a]	126.6 ± .70[b]	33.5 ± .54[a]	8.62 ± .10[a]	25.81 ± .34[a]	32.61 ± .48[a]
		DON	2.66 ± .08[a]	129.8 ± .82[a]	34.3 ± .89[a]	8.37 ± .23[a]	24.37 ± .34[b]	31.50 ± .36[a]

[a,b] Values in the same column, for the same sampling period, and for the same sex with different superscripts are significantly different ($P<0.05$). Values are expressed as mean ± SEM.
ND=Newcastle disease; HI=hemagglutination inhibition; DON = 18mg/kg feed

in female chicks. When compared by sex, we noted that male controls had higher values for hematologic measurements than female controls. It is generally accepted that males of most species have higher hematologic values than females, presumably due to the effects of testosterone. The hematologic effects of feeding DON-contaminated wheat diets to male Leghorn and broiler chicks have been described (Kubena et al. 1985; Harvey et al. 1988; Kubena and Harvey 1988) and the results of the present study support those observations.

Table 2. Mitogen-induced splenic lymphoblastogenesis of Leghorns fed DON-contaminated wheat from 1 day to 9 weeks age (study 2).

	Stimulation Index		
Treatment	3 wks	6 wks	9 wks
Male			
Control	30.9 ± 11.7	12.3 ± 9.4	8.2 ± 3.4
DON	28.5 ± 6.3	21.3 ± 17.4	19.8 ± 12.5
Female			
Control	122.3 ± 15.9	57.3 ± 18.1	38.7 ± 16.1
DON	30.8 ± 4.1*	14.2 ± 7.2**	4.1 ± 1.2**

*P < 0.05; **P < 0.10
n = 3 for each treatment per sampling period; DON = 18 mg/kg feed via contaminated wheat. Values are expressed as mean ± SEM.

In study 3, purified DON fed to broiler chicks induced a significant reduction in the stimulation index of splenocytes of broiler pullets for both replications tested (Table 3). These differences were significant when analyzed by sex and treatment. Hematologic values for broiler chicks did not differ by treatment; however, as in study 2, values for control cockerels were higher than control females (data not shown). In study 4, purified DON did not cause the same suppression of splenocyte blastogenesis in female Leghorn chicks as did the DON-contaminated wheat to pullets of study 2 or the purified DON to broiler pullets in study 3. We cannot offer an explanation for the variances noted in this series of studies; however, it generally accepted that there are sex, species, breed or strain differences of animals to toxicants and that commodities, naturally contaminated with mycotoxins, are more toxic than the same dosages of purified mycotoxin. Perhaps other toxic mycotoxin metabolites or fungal by-products in the naturally contaminated wheat helped contribute to the hematologic toxicity and immunotoxicity. In mice, humoral immunity is suppressed, serum protein fractions altered, B and T cell responses to mitogen reduced, and mortality increased with increasing dosages of DON (Tryphonas et al. 1984; Arnold et al. 1986; Forsell et al. 1986; Robbana-Barnet et al. 1988). Furthermore, diets formulated with naturally occurring DON are more immunotoxic to mice than those containing purified DON (Arnold et al. 1986).

Table 3. Mitogen-induced splenocyte blastogenic response (SI) of chicks fed purified DON for 3 weeks (studies 3 & 4).

	Broilers		Leghorns
Treatment	Rep 1	Rep 2	
Control male	5.21 ± 0.8	4.39 ± 2.0	5.37 ± 0.38
DON male (50 ppm)	5.33 ± 1.0	4.45 ± 0.5	5.27 ± 0.32
Control female	7.05 ± 1.3	10.21 ± 2.6	5.08 ± 0.24
DON female (50 ppm)	5.32 ± 0.9*	5.02 ± 0.7*	5.10 ± 0.22

SI = Stimulation index; stimulated with 25 ug/ml of PHA for 72 hrs.
* $P < 0.05$. n = 3 (Broilers); n = 5 (Leghorns). Values are expressed as mean ± SEM.

While DON has not generally been recognized as overtly toxic to chickens, the results of the present study suggest that we may now include DON as potentially immunotoxic in chicks. In conclusion, the data show that there are sex-related effects of DON on hematologic and immunologic measurements of chickens, and that products naturally contaminated with DON are more toxic than purified DON. Because subtle changes in hematologic or immunologic parameters could affect productivity or disease susceptibility, particularly in young chickens, caution should be exercised when utilizing DON-contaminated feedstuffs to formulate poultry diets.

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