THE EFFECT OF AGE ON THE SUSCEPTIBILITY TO TRI-ORTHO-CRESYL PHOSPHATE INDUCED DELAYED NEUROTOXICITY IN TWO DIFFERENT BREEDS OF CHICKENS[1]

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SUMMARY: The reason for the insusceptibility of young animals to organophosphorus-induced delayed neurotoxicity (OPIDN) remains to be elucidated. The present study was undertaken to determine the influence of age-related changes in hormone concentration on the development of OPIDN in domestic chickens. Growth hormone and testosterone radio immunoassay were utilized to estimate levels of both hormones in this study. It was demonstrated that a relatively fast growing broiler-breed of chicken developed OPIDN when dosed with a single oral dose (500 mg/kg body weight of tri-ortho-cresyl phosphate (TOCP) at six weeks of age while a slower growing layer-breed was not susceptible until 12 weeks of age. The serum growth hormone and testosterone profiles indicated that the growth hormone concentration began to decline in both breeds approximately three weeks before they became susceptible to OPIDN and that testosterone concentrations in the broiler breed increased significantly from one to nine weeks of age; while testosterone concentrations in the layer-breed birds remained relatively constant. TOCP had no significant effect on serum growth hormone in immature chickens.

Key Words: Neurotoxicity, growth hormone, testosterone, organophosphorus.

INTRODUCTION

One of the areas relating to OPIDN which continues to receive attention is the apparent insensitivity of the young of susceptible species to organophosphorus compounds causing delayed neurotoxicity based on clinical and histological observations. Numerous reports suggest that the chicken must be at least 55 to 70 days old at time of exposure in order for the development of OPIDN to occur (1,3,10).

Johnson (11) made the observation that larger species seem to be more susceptible to OPIDN than smaller species. Data reported by Bursian (4) lend support to this observation for avian species in that the small Japanese quail and bobwhite were not susceptible to TOCP-induced delayed neurotoxicity while the larger chicken and pheasant were. If body size and/or rate of growth influence the susceptibility to OPIDN, then it is possible that two different breeds of chickens which have different rates of growth will have different ages of susceptibility to compounds causing OPIDN.

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It is known that growth hormone influences the rate of growth of avian species. Studies by Harvey (9) have demonstrated that plasma GH levels are high in the young bird and then decline to adult levels at a breed-specific age. In the layer-breed birds, the decline in circulating GH begins at 10 to 11 weeks of age while in the faster growing broiler-breed bird, concentrations begin to drop at 4 weeks of age. Since the age at which circulating GH concentrations begin to decline in layer-breed birds corresponds to the age at which this breed is susceptible to OPIDN, it was of interest to test the hypothesis that broiler-breed chickens, which grow at a relatively fast rate and have an early drop in circulating GH concentrations will develop OPIDN at on earlier age than a slower growing layer-breed chicken which has a relatively late decline in circulating GH concentrations.

MATERIALS AND METHODS

Day-old male White Mountain Hubbard (broiler-breed) chicks were purchased from a commercial hatchery and day-old White Leghorn (layer-breed) chicks were obtained from the Department of Animal Science, Michigan State University. Birds were raised in brooder batteries until 4 weeks of age. There were 25 birds per compartment which measured 100x75x63 cm (LxWxH). At 4 weeks of age, birds were transferred to growing

batteries until termination of the experiment. There were 5 broiler-breed birds or 10 layer-breed birds per compartment which measured 98x79x38 cm (LxWxH). Birds were supplied with feed (Purina Chick starter) and water *ad libitum* and exposed to a 16 hour light: 8 hour dark photoperiod.

Beginning at one week of age, 10 birds of each breed were administered either a single oral dose of the delayed neurotoxin tri-o-cresyl phosphate (TOCP) at 500 mg/kg body weight or the corn oil vehicle. At two weeks of age, 10 more birds from each breed received a single oral dose of TOCP or corn oil. This dosing regime continued weekly through 10 weeks of age for the broiler-breed birds and through 12 weeks of age for the layer-breed chickens.

Half of the birds in each group were bled via cardiac puncture 48 hours after dosing between noon an 3p.m. for subsequent hormone analysis. The remaining 5 birds per group were maintained for an additional 19 days. These birds were observed daily from 8 days post TOCP exposure through twenty-one days post-TOCP exposure for the development of clinical signs characteristic of OPIDN. Birds were assessed utilizing an 8-point scale which was modified from Cavanagh (8,9).

Serum for hormone analyses was obtained from the blood collected by cardiac puncture. Two aliquots of serum from each bird were prepared when possible and immediately frozen for subsequent hormone analysis. One aliquot was used for the determination of growth hormone by personnel in the laboratory of Dr. Colin Scanes (Rutgers University, New Brunswick, NJ) and the other aliquot was analyzed for testosterone using radioimmunoassay kit purchased from Sigma Chemical Company.

Hormone data were analyzed using analysis of variance with statements of significance based on $P<0.05$. When the F test was significant, Bonferroni's t-test was used to test for differences between treatment means (8).

RESULTS

The severity of clinical signs typical of OPIDN in broiler and layer-breed chicknes administered a single oral dose of 500 mg TOCP/kg body weight at different ages is summarized in Table 1. Broiler-breed birds were initially susceptible to OPIDN at six weeks of age. Three of the 5 birds dosed at this age developed ataxia which was first apparent at 11 days post-TOCP and progressed to mild ataxia by the end of the 21-day test period. All 5 birds dosed at 7 weeks of age developed OPIDN. Signs were first apparent at 11 days after dosing and by the end of the 21-day test period, the average degree of ataxia was moderate. Birds dosed at 9 and 10 weeks of age were similarly affected with clinical signs beginning at 8 and 9 days post-TOCP, respectively. At the end of the 21-day test period, the broiler-breed birds dosed at 9 weeks of age were severely ataxic and the birds dosed at 10 weeks of age were paralyzed. In contrast to the broiler-breed chickens which were susceptible to OPIDN beginning at 6 weeks of age, layer-breed birds were susceptible at 12 weeks of age. In this group of chickens, clinical signs were first apparent at 14 days post-TOCP, birds were moderately ataxic.

Serum GH concentrations in 1-through 10-week old broiler-an layer-breed birds are presented in Table 2. Serum samples from 11-and 12-week old layer-breed birds were inadvertently discarded before the assay was completed. In both breeds, serum GH concentrations declined significantly with age as determined in the corn oil-treated birds. In broile-breed birds, a sharp decline in GH concentration occurred at 3 weeks of age while in layer-breed birds, the decline was more gradual over the 10-week period. The administration of TOCP had no significant effect on serum GH concentrations except in 1-week old broiler-breed birds where the organo-phosphate-treated group had a significantly lower mean concentration than the control group.

Serum testosterone concentrations in broiler-and layer breed chickens from 1 to 9 weeks of age are presented in Table 3. Serum samples from 10-week old broiler-breed birds and 11-and 12-week old layer-breed birds were inad-

Table 1: Average degree of ataxia in broiler and layer breeds of chickens administered a single oral dose of 500 mg TOCP/kgm body weight at difference ages.

			Days post-TOCP adminitration													
	Age (wks)	n[b]	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Broiler	5	5/5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	6	3/5	0	0	0	0.3	0.7	0.7	0.7	0.7	0.7	0.7	1.0	2.3	2.7	2.7
	7	5/5	0	0	0	0.6	0.6	1.3	1.8	1.8	2.6	2.6	3.8	3.8	3.8	3.8
	9	4/5	1.8	2.8	3.8	3.5	3.5	4.5	4.8	5.5	5.5	5.5	6.0	6.3	6.3	6.3
	10	5/5	0	0.8	1.8	1.8	6.0	7.6	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Layer	11	5/5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	5/5	0	0	0	0	0	0	1.0	1.6	2.4	2.4	2.6	3.0	3.6	3.6

a. Mean degree of ataxia based on the number of birds which were ataxic during the 21 day observation period. Birds were scored on an 8-point scale which was modified from Cavanagh (5,6) where 0=normal; 1-2=slight but definite ataxia; 3-4=ataxia but without serious incapacitation; 5-6=marked ataxia with an inability to maintain an upright stance for any length of time; 7-8=total inability to rise or walk.
b. Number of ataxic birds/number of birds dose.

vertently discarded before the assay was completed. In the broiler-breed birds, there was an age-related increase in testosterone concentrations over the 9 week period represented while testosterone concentrations in the layer-breed birds did not significantly vary with age. Eight-and 9-week old broilers treated with 500 mg TOCP/kg body weight had significantly higher serum testosterone concentrations when compared to their respective controls. Serum testosterone concentrations were not significantly affect by TOCP administration in the layer-breed birds.

DISCUSSION

The present study indicates that the breed of chicken influences the age of susceptibility to the delayed effects of TOCP (Table 1). Broiler-breed birds began having clinical signs characteristic of OPIDN when dosed with TOCP at 6 weeks of age. As successively older chickens were dosed with TOCP, clinical signs were apparent earlier and were more severe at the end of the 21-day test period. In contrast, the layer-breed birds did not begin to show clinical signs when dose with TOCP until 12 weeks of age which is consistent with earlier reports (7). The influence of breed on the development of OPIDN has not been systematically studied. Indeed, in many studies the breed of chicken is not specified, leaving one to assume that the typical layer-breed bird (White Leghorn) is the experimental animal.

Previous studies have indicated that the immature chicken is not susceptible to the delayed effects of certain organophosphorus compounds. For example, Barnes and Denz (1) reported that 4 chicks (Rhode Island Red X Light Sussex) which received 10 successive weekly subcutaneous doses of 1 mg DFP/kg body weight beginning at 2 weeks of age did not develop paralysis which typically would have been observed in adult birds administered a single dose of 1 mg DFP/kg body weight. In a similar experiment, Johnson and Barnes (10) injected chicks (various unspecified breeds) subcutaneously with single dose of 2 to 5 mg DFP/kg body weight from 7 to 49 days of age and observed no delayed effects. However, the susceptibility of the chicks to the delayed effects of DFP progressively increased when the compound was injected between 60 and 100 days of age. Bondy (3) administered single oral doses of 1000 mg TOCP/kg body weight to chicks (incross strain) of varying ages. They reported that TOCP was not effective in producing OPIDN when administered at 10,20,30,40 or 50 days of age but was effective if administered at 72 and 100 days of age.

Baron (2) has suggested that the resistance of the young bird to the delayed effects of orally administered TOCP is due to poor absorption of the compound through the gastrointestinal tract. He cited unpublished data indicating that if TOCP were administered to 4-week old chicks (unspecified breed) by intraperitoneal

Table 2: Serum growth hormone (GH) concentrations (ng/ml) in broiler-and layer-breed chickens 48 hours after administration of 500 mg TOCP/kgm body weight at different ages.

Breed	Age of dosing with TOCP (wks)	Treatment	
		Control	TOCP
Broiler	1	398 ± 79.6(5)[a]	76 ± 79.6 (4)[b]
	2	391 ± 79.6(5)	230 ± 79.6(5)
	3	108 ± 79.6(5)	234 ± 89.0(4)
	4	143 ± 79.6(5)	139 ± 102.7(3)
	5	114 ± 79.6(5)	285 ± 79.6(5)
	6	76 ± 79.6(5)	102 ± 102.7(3)
	7	84 ± 79.6(5)	59 ± 79.6(5)
	8	41 ± 79.6(5)	54 ± 79.6(5)
	9	58 ± 89.0(4)	58 ± 79.6(5)
	10	104 ± 89.0(4)	205 ± 102.7(3)
Layer	1	550 ± 89.0(4)	172 ± 102.7(3)
	2	546 ± 79.6(5)	728 ± 79.6(5)
	3	542 ± 89.0(4)	361 ± 79.6(5)
	4	418 ± 89.0(4)	731 ± 102.7(3)
	5	76 ± 79.6(5)	312 ± 89.0(4)
	6	338 ± 79.6(5)	304 ± 79.6(5)
	7	118 ± 79.6(5)	239 ± 79.6(5)
	8	214 ± 79.6(5)	368 ± 79.6(5)
	9	243 ± 79.6(5)	351 ± 79.6(5)
	10	210 ± 102.7(3)	210 ± 79.6(5)

a. Mean ±standard error. Numbers in parentheses refer to sample size.

b. Significantly different from control value at same age.

injection, then clinical signs characteristics of OPIDN could be observed while if TOCP were administered orally, clinical signs were evident only in birds 12 weeks of age or older. In contrast to Baron's study, Olson and Bursian (12) demonstrated that 4- week old White Leghorn chicks were not susceptible to TOCP or its neuroactive metabolite, o-tolyl saligenin phosphate, regardless of the route of administration. This suggested that the resistance of the young chicken to the delayed effects of organophosphorus compounds is due to factors other than poor absorption of the compound through the gastrointestinal tract.

Given the fact that breed does influence the age of susceptibility to the delayed effects of TOCP, there must be physiological differences between the layer-breed bird and broiler-breed bird which account for the difference in age susceptibility. One obvious difference between the 2 breeds is rate of growth. For example, at 6 weeks of age, the average body weight of a broiler -breed male is 1300 gms while the average weight of a White Leghorn cockerel (a laying-breed bird) is 600 gms. Harvey (9) reported that the difference in growth rate between broiler-breed and layer-breed birds was related to the difference in circulating growth hormone concentrations. Results of the present study (Table 2) confirm those reported by Harvey (9) and Scanes (13) in that there was a negative relationship between age and GH concentrations. They reported that GH concentrations in layer-breed were highest from 3 to 9 weeks of age with a subsequent decline to adult concentrations at about 12 weeks of age. In broiler-breed birds, the same phenomenon occurred, but the major drop in GH concentration was around three weeks of age. In both breeds, the decline in GH concentration is related in some way to the age of susceptibility to OPIDN.

The other endocrine parameter which was examined in relation to the age at which OPIDN first developed was serum testosterone concentration. In the broiler-breed birds, serum testosterone increased over the 9-week period it was analyzed, while in the layer-breed birds, testosterone concentration remained relatively constant over the 9-week period (Table 3). As with growth hormone, it is possible that increasing concentrations of testosterone in the broiler-breed birds are contributing to their susceptibility to OPIDN at an earlier age when compared to the layer-breed chickens.

In conclusion, the results of the present study indicate that broiler-breed birds were susceptible to the delayed effects of TOCP at 6 weeks of age as opposed to layer breed birds which were susceptible beginning at 12 weeks of age. The differences in growth rate and the profiles of serum growth hormone and testosterone between the 2 breeds suggest that these endocrine parameters warrant further investigation in relation to OPIDN.

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