Changes in Mineral Composition of Eggshells from Black Ducks and Mallards Fed DDE in the Diet.

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Introduction

DDE, a metabolite of DDT, is a persistent chemical that has become widespread in the environment within the last three decades. It has been associated with reproductive failures due to eggshell thinning and cracking in many avian species (1,2,3). Experimental studies have shown that p,p' DDE in food induces thinning in eggshells of sparrow hawks (4), mallards (5), and black ducks (6). Decreases in eggshell thickness have been attributed to a reduction in the supply of calcium available for deposition by the shell gland (7). Furthermore, calcium carbonate accounts for most of the thickness of an eggshell (8). Bitman et al. (9) found that the shells of eggs from coturnix quail fed diets containing DDT were not only significantly thinner, but contained a smaller percentage of shell calcium than those from undosed birds. There is a paucity of information concerning concentrations of other minerals in eggshells from birds exposed to organochlorine compounds.

Material reported in this paper was obtained in conjunction with studies to determine the extent to which DDE affects reproduction of captive black ducks (6) and mallards (10). In these studies, the ducks were maintained, beginning several months prior to laying, on commercial duck mash; it was either untreated or it contained p,p' DDE at rates of 10 or 30 ppm (dry weight) for the black ducks, and rates of 1, 5, and 10 ppm (dry weight) for the mallards. A sample of eggshells from those ducks was analyzed to determine if DDE in the diet could be associated with altered mineral concentrations in the eggshells.

Procedures

Shells of the third egg laid by each of 37 black ducks, and 33 assorted eggs from mallards were selected for mineral analyses. Eggs were opened at the equator, the contents were removed, and the shell was washed in water and air-dried before shipment to WARF Institute, Inc., for analyses. Eggshell minerals were determined by direct reading emission spectroscopy according to methods of Christensen et al. (11).

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Data were evaluated by analysis of variance, and significant differences among means were determined by Kramer's extension for unequal subclasses of Duncan's multiple range test. Correlation coefficients were obtained among black duck eggshell minerals for each dosage group.

Results and Discussion

Concentrations of 14 mineral elements in the eggshells are shown in Table 1. The elements phosphorus, potassium, calcium, magnesium, and sodium are expressed in percentage of total dry weight since these minerals are present in relatively large amounts (1 percent equals 10,000 ppm). All other elements are expressed in ppm dry weight.

Shells of eggs laid by black ducks fed DDE in their diets contained significantly ($P<0.01$) higher percentages of magnesium, significantly lower percentages of strontium, and significantly lower percentages of barium than did shells of eggs laid by black ducks fed untreated diets. The average percentage of calcium was lower in shells of eggs from DDE-dosed ducks with the difference approaching significance ($P=0.05$). The average percentages of sodium and copper in shells of dosed ducks were higher but again the difference only approached significance. For the eggshells from mallards dosed with 1, 5, and 10 ppm (dry weight) DDE, there was a highly significant increase in the average percentage of magnesium in the eggshells from both the 5 and 10 ppm dosage groups as compared to the control group. There were no significant decreases in strontium or barium levels, but the average percentages of aluminum in eggshells from both the 5 and 10 ppm dosage groups were significantly lower. The average percentage of calcium in eggshells from mallard ducks fed 10 ppm DDE in the diet was significantly lower than the levels in eggshells from control mallards. The average percentage increase in sodium approached significance for eggshells in the 10 ppm DDE ($P=0.05$) dosage group. These percentage changes should be interpreted cautiously. For example, the percentage "increase" in magnesium could result from the base of the percentage (i.e., weight of shell) being reduced because of the lack of deposition of another mineral such as calcium.

Correlation tests for the eggshells from black ducks were made for all possible pairs of the minerals for each treatment. Of the 273 tests, there were 35 significant correlations (Table 2). Some mineral correlations occurred only in one of the dosage groups, while two correlations, potassium with chromium and aluminum with chromium, were common to all dosage groups.

TABLE 1

Concentrations of selected minerals in eggshells from black ducks and mallards fed DDE in the diet (dry weight).

Mineral	Average concentrations of minerals in eggshells (dry weight)						
	Black Ducks			Mallards			
	DDE in Diet						
	Untreated (n=13)	10 ppm (n=14)	30 ppm (n=10)	Untreated (n=11)	1 ppm (n=6)	5 ppm (n=7)	10 ppm (n=9)
	pph						
Phosphorus (P)	< 0.05	< 0.05	< 0.05	< 0.19	< 0.11	< 0.14	< 0.15
Potassium (K)	1.04	1.03	1.01	1.81	1.82	1.84	1.80
Calcium (Ca)	34.09	32.57	32.98	35.15	35.40	34.98	34.02*
Magnesium (Mg)	0.218	0.276**	0.295**	0.142	0.143	0.170**	0.172**
Sodium (Na)	0.232	0.251	0.243	0.235	0.233	0.243	0.251
	ppm						
Aluminum (Al)	1259.2	1235.7	1258.0	1069.0	1098.0*#	1052.0*	1032.0*
Barium (Ba)	48.2	35.2*	33.4*	36.9	37.2	36.7	32.1
Iron (Fe)	156.0	152.5	125.0	67.1	62.0	67.7	74.6
Strontium (St)	237.7	157.4**	159.0**	281.0	320.0	300.0	310.0
Boron (B)	13.8	16.1	10.8	10.3	9.8	9.9	10.8
Copper (Cu)	36.8	42.4	42.5	37.4	35.0	34.9	35.6
Zinc (Zn)	11.4	13.8	10.3	11.7	11.0	10.4	11.3
Manganese (Mn)	< 4.0	< 4.0	< 4.0	< 4.0	< 4.0	< 4.0	< 4.0
Chromium (Cr)	45.8	45.5	32.8	57.27	57.50	57.71	58.77

n = Number of eggshells analyzed.
* = Difference from control for respective species significant ($P<0.05$).
** = Difference from control for respective species highly significant ($P<0.01$).
# = This significant difference probably an artifact because of small sample and high values (exceeded controls) for two shells.

TABLE 2

Correlation coefficients among minerals in eggshells from black ducks fed DDE in ppm dry weight

Minerals Correlated	Mineral correlation coefficients for diets		
	Untreated	10 ppm DDE	30 ppm DDE
K/Ca	0.62*	0.60*	
Al	0.77**	0.55*	
Cr	0.94**	0.54*	0.64*
Zn		-0.61*	
Ca/Al	0.73**	0.62*	
Cr	0.65*		
Ba		0.63*	
Zn		-0.62*	
Mg/Na	0.65*		
Ba	0.66*		
Sr	-0.64*	-0.67*	
Fe		-0.64*	
Cu		-0.60*	
Na/Al		-0.58*	-0.64*
Ba		-0.61*	
Zn			0.76*
Al/Ba		0.58*	0.83**
Cr	0.79**	0.81**	0.90**
Zn		-0.87**	
Ba/Zn		0.57*	
Fe			0.64*
Sr			0.64*
Cr			0.81**
Cr/Zn		-0.88**	
Sr			0.71*
Fe/Zn	0.60*		

* = $P<0.05$
** = $P<0.01$

Correlation coefficients were also determined for eggshell thickness and shell minerals among the eggshells from black ducks. The only significant correlations between the eggshell thickness and eggshell minerals occurred in the control group. In this group, higher levels of both magnesium and sodium were associated with thinner eggshells.

Changes in mineral contents of various tissues have been noted in rats (12,13), clams (14), fish (15), and plants (16) that have been exposed to various chemicals. The changes may be of no serious consequence, but Lawrence et al. (12) suggest that trace metal shifts may not only result from, but contribute to, the toxic state (in a pesticide intoxicated organism) and as such could provide a basis for the detection and evaluation of intoxication before gross physiological responses are manifested.

Decreases in percentage of calcium, the major eggshell mineral, have been associated with decreases in eggshell weight, thickness, and strength (8,17). The importance of some of the other trace minerals in eggshell formation in unknown.

Minerals such as calcium and phosphorus must be present in the egg in adequate amounts and in the proper ratio to be normally assimilated by the embryo (18). Similarly, eggshell minerals may need to be in adequate concentrations and in proper ratios to provide sufficient shell strength. In this study, eggshells from DDE-fed black ducks were 18-24% thinner than eggshells from undosed black ducks and had significantly more cracks (6). Further research is needed to show the means by which some of these mineral shifts occur and their significance.

Summary

Diets containing 10 and 30 ppm (dry weight) DDE were fed to black ducks, and diets containing 1, 5, and 10 ppm (dry weight) DDE were fed to mallards. Among the results were the following changes in black duck eggshell composition: (a) significant increase in the percentage of Mg, (b) significant decreases in Ba and Sr, (c) increases (which approached significance) in average percentage of eggshell Na and Cu, (d) a decrease in shell Ca which approached significance, (e) patterns of mineral correlations which in some instances were distinct to dosage groups, and (f) inverse correlations in the control group between eggshell thickness Mg and Na.

Changes in mallard eggshells were: (a) significant increase in percentage of magnesium at 5 and 10 ppm DDE, (b) significant decrease in Al at 5 and 10 ppm DDE, (c) a significant decrease in Ca from eggshells from the 10 ppm DDE group, and (d) an increase in average percentage of Na in eggshells from DDE dosed ducks which approached significance.

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