Bull. Environm. Contam. Toxicol. 26, 328-336 (1981)

South Louisiana Crude Oil or DDE in the Diet of Mallard Hens: Effects on Egg Quality

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Egg quality must ultimately be measured in terms of survival of successfully hatched progeny. The size and composition of the egg are directly related to the size and composition of the hatchling and influence the hatchling's ability to survive, especially in precocial birds. In this paper, we examine the effects of two environmental pollutants, crude oil or DDE, on the size and composition of mallard (Anas platyrhynchos) eggs.

METHODS AND MATERIALS

We provided 67 mallard hens with free access to untreated food and water beginning 29 January 1979. On 8 February 1979, two groups of 21 hens were assigned at random to receive either a diet containing 10 ppm DDE (Patuxent Wildlife Research Center) or a diet containing South Louisiana crude oil (SLCO) (Amer. Pet. Inst. Ref. oil 2) in a concentration of 2%. The remaining 25 hens continued to receive untreated food and served as controls. Further details concerning maintenance of experimental animals can be found in VANGILDER and PETERLE (1980). Eggs were collected daily, weighed, measured, and identified by hen and date. Alternate eggs from each hen were kept for analyses. Eggs not kept for analyses were incubated to determine fertility, hatchability, and survivorship of newly hatched young (VANGILDER and PETERLE 1980). Each collected egg was hard-boiled and separated into its three components: shell, yolk, and albumen. Wet weights were determined for each component. Shells were dried for 24 h at 100° C to determine dry weight. The first five eggs from each of five hens from each treatment group were selected for further analyses. Yolks were analyzed for water, protein, lipid, and gross energy; albumens for water, protein, and gross energy. Protein content was determined by Kjeldahl nitrogen determination (A.O.A.C. 1975); lipid content by the method of FOGERTY et al. (1971). Gross energy determinations were made using a Parr adiabatic bomb calorimeter and were corrected for fuse-wire ignition and acid production.

RESULTS

Dietary treatment of hens with SLCO but not DDE, altered the mean size and composition of eggs (Table 1).

0007-4861/81/0026-0328 $01.80

TABLE 1

Characteristics of eggs laid by mallard hens fed either a control, SLCO (2% W/W) or DDE (10ppm) diet. Tabled values are the mean ± 1 standard error. Means are in grams unless otherwise specified.

Characteristic	Diet		
	Control N=25	SLCO(2%) N=25	DDE(10ppm) N=25
Egg weight	58.45±1.32	53.21±0.72*	59.24±0.86
Egg length (cm)	5.76±0.08	5.59±0.05*	5.89±0.04
Egg breadth (cm)	4.23±0.03	4.13±0.02*	4.22±0.02
Shell weight	5.90±0.15	5.06±0.12*	5.91±0.08
Yolk weight	21.69±0.52	18.33±0.38*	21.04±0.41
Albumen weight	30.87±0.85	29.83±0.49	32.30±0.47
Shell water weight	0.63±0.02	0.74±0.03*	0.69±0.02
Yolk water weight	10.26±0.27	8.45±0.15*	9.81±0.24
Albumen water weight	26.78±0.72	25.95±0.47	28.01±0.42
Dry shell weight	5.26±0.14	4.32±0.13*	5.21±0.07
Dry yolk weight	11.42±0.26	9.88±0.26*	11.23±0.20
Dry albumen weight	4.09±0.13	3.88±0.06	4.29±0.07
Yolk protein weight	3.29±0.08	2.83±0.06*	3.25±0.05
Albumen protein weight	3.58±0.13	3.34±0.06	3.66±0.07
Yolk lipid weight	7.07±0.19[a]	6.20±0.18*	6.79±0.16[a]
Yolk energy (Kcal)	88.39±2.04	76.75±2.08*	87.00±1.54
Albumen energy (Kcal)	20.14±0.66	18.89±0.35	21.09±0.36

[a] N=21

* $P<0.05$ Treated vs control diet (DUNNETT 1964)

Hens fed the SLCO diet (SLCO hens) laid eggs which were significantly lighter and smaller than those laid by hens fed the control diet (control hens) ($P<0.05$). Eggs laid by SLCO hens (SLCO eggs) also had lighter shells and contained less yolk than did eggs laid by control hens (control eggs) ($P<0.05$). Shells from SLCO eggs contained more water than did those from control eggs ($P<0.05$). Yolks from SLCO eggs contained less water than did those from control eggs ($P<0.05$). The density of protein (28.85±0.16 g/100 g dry yolk, n=75), lipid (61.88±0.27 g/100 g dry yolk, n=67), and energy in the yolks (774.8±0.77 Kcal/100 g dry yolk, n=75), and albumens (490.1±1.14 Kcal/100 g dry albumen, n=75) was similar for all eggs ($P>0.05$). Because the yolks in SLCO eggs were smaller

than those of control eggs, they contained less total protein, lipid, and energy ($P<0.05$) (Table 1).

The data presented in Table 1 examine only absolute differences in egg characteristics. Pollutant-induced changes in the relative proportions of components within the egg could also influence egg quality. To detect proportionate changes in egg characteristics, each component in Table 1 (except length and breadth) was regressed on egg weight for all eggs. The regression equations were then compared to detect differences in error variance, slope, and elevation (NETER AND WASSERMAN 1974, SNEDECOR and COCHRAN 1967) (Table 2).

The regressions of shell weight (wet or dry) on egg weight for control and DDE eggs were not different. The weight of shells (wet or dry) from SLCO eggs was not correlated with egg weight. The weight of water in the shells of DDE eggs was significantly but poorly related to egg weight.

The fresh weight of yolk in all eggs increased at a similar rate with egg weight but yolks from SLCO and DDE eggs were proportionately smaller than yolks of control eggs. Yolk water weight in all eggs also increased at a similar rate with egg weight but the weight of yolk water in SLCO and DDE eggs was proportionately less than in control eggs. The regressions of dry yolk weight, yolk lipid weight, and yolk energy on egg weight for control and DDE eggs were not different, but dry yolk weight, yolk lipid weight, and yolk energy in SLCO eggs increased at a more rapid rate with egg weight. The weight of yolk protein in eggs from all groups increased at a similar rate with egg weight. The weight of yolk protein in SLCO eggs was proportionately less than the weight of yolk protein in control eggs, but DDE eggs did not differ in this respect.

The fresh weight of albumen in all eggs increased at a similar rate with egg weight but albumens in SLCO and DDE eggs were proportionately larger than those in control eggs. The weight of albumen water in all eggs also increased at a similar rate with egg weight but the weight of albumen water in SLCO and DDE eggs was proportionately larger than that in control eggs. The regressions of dry albumen weight on egg weight for all groups were not different. The weight of albumen protein in SLCO eggs was not related to egg weight. The regressions of albumen protein weight on egg weight for control and DDE eggs were not statistically compared because of non-homogeneous residual variation. The regressions of albumen energy on egg weight for all groups were not different.

Component and egg weight data were also converted to logarithms and regression analysis was performed. A slope of the regression between the logarithm of a component and the logarithm of egg weight that is significantly different from 1 indicates that the proportion of the component changes systematically with egg weight (RICKLEFS et al. 1978). Among group comparisons of the

regressions of each component on egg were not done. Instead, the slope of each regression was tested to determine whether it significantly differed from 1. In most cases each component increased in direct proportion to egg weight for each group. Exceptions are discussed below. The regressions of the logarithm of shell weight (wet or dry) and the logarithm of albumen protein weight on the logarithm of the weight of SLCO eggs were not significant. The regressions of the logarithm of shell water weight on the logarithm of the weight of control or SLCO eggs were not significant and the logarithm of the weight of shell water was only poorly correlated with the logarithm of the weight of DDE eggs. The proportion of shell (wet or dry) in DDE eggs decreased with increasing egg weight (slope $\pm$ 1 SE wet = 0.6938 $\pm$ 0.1399, dry = 0.6396 $\pm$ 0.1539; $P<0.05$). The proportion of lipid in the yolks of SLCO eggs increased with increasing egg weight (slope $\pm$ 1 SE = 1.6978 $\pm$ 0.2585; $P<0.05$).

DISCUSSION

Eggshell quality (porosity and thickness) influences gas and water exchange between the environment and the developing embryo and thus, influences hatchability and hatchling viability (COOKE 1979, FOX 1976). The weight of shells of SLCO eggs was lighter than that of control eggs and was not correlated with egg weight. The mean shell weight of DDE eggs and the linear relationship between shell and egg weights were not different from that of control eggs, but the weight of shells of DDE eggs did not increase in direct proportion to egg weight. Eggshell thickness was reduced by 5 and 16% for DDE and SLCO eggs, respectively, when compared with that for control eggs (VANGILDER and PETERLE 1980). The quality of eggshells was reduced by SLCO and DDE. The porosity and mineral composition of avian eggshells has also been found to be altered by DDT contamination (COOKE 1979, FOX 1976, LONGCORE et al. 1971).

AR and RAHN (1980) present evidence suggesting that an egg of given mass must initially contain a fixed concentration of water and lose a fixed amount of water during incubation in order for the egg to hatch. The albumen of SLCO and DDE eggs of any given size contained more water than did albumen of control eggs. Hatchability of SLCO and DDE eggs was 46 and 64%, respectively; control eggs 85% (VANGILDER and PETERLE 1980).

The observed changes in both shell quality and albumen water content are probably the result of pollutant-induced changes in shell gland function. The size and number of pores in avian eggshells depend in part upon "plumping fluid" which passes into the egg albumen as the shell is formed in the shell gland (SIMKISS 1980).

The mean size of yolks and thus, the protein, lipid, and energy content of SLCO eggs were reduced when compared with that of control eggs. In addition, the lipid content of yolks from SLCO eggs did not increase in direct proportion to egg weight. HOLMES

TABLE 2

Statistics for the regression of each egg component on egg weight for control, DDE, and SLCO eggs. Statistical comparisons of treated vs control egg data regressions were made if error variances were homogeneous. The slopes of treated vs control regressions for each component were compared first. A regression with a slope statistically different from the control regression precluded further analysis. If the slopes were not different, a common slope was calculated and intercepts were compared by adjusting the mean of the component for each group to the mean egg weight for the groups combined. Where applicable, the difference between the adjusted mean of a treatment component and a control component ± 95% confidence limits were calculated. Each regression is based on 25 observations. Statistical comparisons of slopes and intercepts were made with α=0.05.

Component	Treatment Group	Intercept	Slope ± 1 SE	R^2	Significance	Common Slope	Adjusted Mean	Difference ±95% CL
Shell	CONTROL	0.2815	0.0961±0.0118	0.74	0.0001			
weight	SLCO	-	-	-	NS[b]	0.0879[d]	NS	
(wet)	DDE	1.7377	0.0704±0.0132	0.55	0.0001			
Yolk	CONTROL	3.0683	0.3185±0.0493	0.64	0.0001		21.15	
weight	SLCO	-3.6066	0.4122±0.0669	0.62	0.0001	0.3609	19.69*	1.46±0.88
(wet)	DDE	-4.0615	0.4237±0.0449	0.79	0.0001		20.22*	0.93±0.81
Albumen	CONTROL	-3.3499	0.5854±0.0546	0.83	0.0001		30.03	
weight	SLCO	-1.2038	0.5832±0.0737	0.73	0.0001	0.5654	31.95*	-1.92±0.95
(wet)	DDE	2.3238	0.5060±0.0449	0.85	0.0001		31.01*	-0.98±0.87
Shell	CONTROL	-	-	-	NS			
water	SLCO	-	-	-	NS		NC[c]	
weight	DDE	-0.0816	0.0131±0.0052	0.21	0.0196			
Yolk	CONTROL	0.9646	0.1591±0.0274	0.59	0.0001		9.96	
water	SLCO	1.2093	0.1360±0.0317	0.44	0.0003	0.1713	9.09*	0.87±0.51
weight	DDE	-3.4912	0.2244±0.0341	0.65	0.0001		9.42*	0.54±0.47

TABLE 2 (cont.)

Component	Treatment Group	Intercept	Slope ± 1 SE	R^2	Significance	Common Slope	Adjusted Mean	Difference ±95% CL
Albumen	CONTROL	-2.8237	0.5064±0.0438	0.85	0.0001		26.04	
water	SLCO	-2.7070	0.5385±0.0768	0.68	0.0001	0.4984	27.82*	-1.78±0.85
weight	DDE	1.2650	0.4515±0.0396	0.85	0.0001		26.88*	-0.84±0.78
Dry shell	CONTROL	-0.1153	0.0920±0.0118	0.73	0.0001			
weight	SLCO	-	-	-	NS	0.0808[d]	NS	
	DDE	1.8193	0.0573±0.0133	0.45	0.0003			
Dry yolk	CONTROL	2.1038	0.1595±0.0251	0.64	0.0001			
weight	SLCO	-4.8159	0.2762±0.0467*	0.60	0.0001	0.1692[d]	NS	
	DDE	-0.5703	0.1992±0.0225	0.77	0.0001			
Dry	CONTROL	-0.5262	0.0791±0.0136	0.60	0.0001			
albumen	SLCO	1.4987	0.0447±0.0156	0.26	0.0088	0.0649	NS	
weight	DDE	1.0588	0.0545±0.0137	0.41	0.0006			
Yolk	CONTROL	0.3110	0.0510±0.0076	0.66	0.0001		3.21	
protein	SLCO	-0.7607	0.0674±0.0093	0.70	0.0001	0.0540	3.03*	0.18±0.13
weight	DDE	0.2129	0.0513±0.0054	0.80	0.0001		3.13	NS
Albumen	CONTROL	-0.9091	0.0768±0.0139	0.57	0.0001			
protein	SLCO	-	-	-	NS		NC	
weight	DDE	-0.0021	0.0618±0.0090	0.63	0.0001			
Yolk lipid	CONTROL[a]	0.7280	0.1075±0.0157	0.71	0.0001			
weight	SLCO	-4.3429	0.1982±0.0327*	0.61	0.0001	0.1130[d]	NS	
	DDE[a]	-0.5757	0.1255±0.0256	0.56	0.0001			
Yolk	CONTROL	15.8894	1.2404±0.1918	0.65	0.0001			
energy	SLCO	-39.5983	2.1864±0.3884*	0.58	0.0001	1.3193[d]	NS	
(Kcal)	DDE	-5.2670	1.5574±0.1833	0.76	0.0001			

TABLE 2 (cont.)

Component	Treatment Group	Intercept	Slope ± 1 SE	R^2	Significance	Common Slope	Adjusted Mean	Difference ±95% CL
Albumen energy (Kcal)	CONTROL	-2.1984	0.3821±0.0675	0.58	0.0001			
	SLCO	5.0358	0.2603±0.0861	0.28	0.0060	0.3277	NS	
	DDE	5.7406	0.2590±0.0678	0.39	0.0009			

[a] N = 21

[*] $P < 0.05$ treated vs control diet

[b] Not significant

[c] No comparison

[d] Common slope for control and DDE only

et al. (1978) found that mallard hens fed 3% SLCO in their diet for 150 days had smaller ovaries and oviducts than those fed a control diet. Follicular mass and the proportion of non-atretic follicles were also reduced. Japanese quail (Coturnix coturnix) and chickens fed single doses of SLCO produced eggs with altered yolk structure (GRAU et al. 1977). Representative aromatic hydrocarbons of SLCO in the diet of male mallards resulted in a significant increase in liver weight, plasma clearance of indocyanine green dye, and hepatic blood flow (PATTON and DIETER 1980). The increased physiological demand placed on the liver of female mallards during vitellogenesis (GURAYA 1978) combined with hepatic stress previously induced by petroleum ingestion could partially explain the observed alterations in yolk size, structure, and composition.

Ducklings hatching from either SLCO or DDE eggs and subjected to starvation and temperature stress exhibited reduced thermoregulatory ability and survivorship (VANGILDER and PETERLE 1980). The mean size of SLCO eggs, but not DDE eggs, that produced ducklings was significantly smaller than that of control eggs ($P<0.05$).

Pollutant-induced changes in the physical characteristics of the egg as well as physiological changes in the embryo induced by the presence of pollutants in the egg probably interact to produce the observed reductions in egg hatchability, thermoregulatory ability and survival of ducklings.

ACKNOWLEDGEMENTS

This project was supported primarily through a grant from the U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center (USDI 14-16-0009-78-973). S. Cameron provided assistance with the research.

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