Dieldrin and p,p'—DDT Effects on Egg Production and Eggshell Thickness of Chickens[1]

by K. L. DAVISON
U. S. Department of Agriculture
Metabolism and Radiation Research Laboratory
Animal Science Research Division, ARS
Fargo, N. D. 58102
and
J. L. SELL
Animal Science Department
North Dakota State University
Fargo, N. D. 58102

A general, sometimes dramatic, decline has occurred in shell thickness of eggs of many species of birds since the end of World War II (1,2). In some cases, eggshells have been reported to be more than 25% thinner than those collected prior to that time (3, 4). Since the eggshell thinning was coincident with the beginning of widespread use of DDT and other chlorinated hydrocarbon insecticides, and since residues of these insecticides were found in these eggs, the chlorinated hydrocarbon insecticides have been associated with this thinning.

Several experiments have been conducted to determine effects of feeding dieldrin, DDT, DDD or DDE on eggshell thickness of various species of birds (5-12). In all cases, the authors concluded that these insecticides caused thinning of eggshells, but in no cases were the changes in shell thickness in these experiments as dramatic as have apparently occurred in the wild. The outcome of these experiments was essentially the same irrespective of whether raptorial, aquatic or gallinaceous birds were used.

Our objectives were to study effects of feeding high levels of dieldrin or p,p'-DDT on egg production and eggshell thickness in chickens.

Methods

Sixty White Leghorn hens, 28 weeks old, were housed in individual wire cages. All hens consumed 90 g daily of a commercial nutritionally balanced laying hen diet containing 3.2% calcium. Individual hens served as the experimental units.

Following a two-week adjustment period, the hens were randomly allotted to five treatment groups of 12 hens. Treatments consisted

---

[1] Published with the approval of the director of the North Dakota Agricultural Experiment Station as Journal Article No. 301.

of dieldrin[2] and p,p'-DDT[3] added to the diet as follows: (1) control, (2) 10 ppm dieldrin, (3) 20 ppm dieldrin, (4) 100 ppm p,p'-DDT, and (5) 200 ppm p,p'-DDT. The insecticides were dissolved in 400-500 ml of acetone and premixed into 2 kg of the respective diets. These premixes were then mixed into enough diet for the entire 12-week experimental period. The diets were stored in polyethylene bags in covered steel cans until used. Assays of the control diet by electron capture, gas-liquid chromatography (13) revealed less than 0.01 ppm dieldrin and 0.1 ppm DDT.

Eggs were collected, dated and weighed to the nearest 0.1 g. The experimental protocol called for shell thickness, shell calcium and dried egg contents to be measured on all eggs laid on days 1, 14, 28, 42, 56, 70 and 84, and on all eggs laid in clutches nearest days 1, 28, 56 and 84. Clutch is defined as eggs laid on successive days. Freeze-dried egg contents were weighed to the nearest 0.01 g.

Thickness of the eggshell (without membranes) was measured at two locations on the waist of the egg with a micrometer. The micrometer was equipped with convex contact points and a numerical scale that could be read to 0.01 mm and estimated to 0.001 mm. Oven-dried eggshell was weighed and ashed, and the ash dissolved in HCl. Calcium was determined by direct EDTA titration using hydroxynapthol blue as indicator according to the general procedures of Hurwitz and Griminger (14), as slightly modified by Hurwitz (15).

The data were analyzed statistically by least squares analysis of variance, with significance assessed at probabilities of 5% or less. Egg production and egg weights were examined statistically for total eggs laid, eggs laid per clutch, eggs laid each week and eggs laid in 12-day periods. Eggshell characteristics were examined at the intervals mentioned above. The statistical model for the analysis of variance of data on eggshell thickness is shown in Table 1.

Results

Data summarized for the entire 12-week period are shown in Table 2. Body weight of the hens did not vary appreciably during

---

[2] Supplied as technical dieldrin, but enough was used to supply 10 or 20 ppm of the active ingredient 1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene.

[3] p,p'-DDT, 1,1,1-trichloro-2, 2-bis (p-chlorophenyl) ethane, 99% pure, as stated by the manufacturer (Aldrich Chemical Co., Inc., lot No. 100571).

TABLE 1

STATISTICAL ANALYSIS OF EGGSHELL THICKNESS OF EGGS COLLECTED IN CLUTCHES AT DAYS 28, 56 and 84[a]

Source of Variation	Degrees of Freedom	Mean Square
Total	812	
MU[b]	1	.000655
Diets (Insecticides)	4	.001575
Hens within Diets (Error A)[c]	54	.004003
Time	2	.000906
Diet X Time	8	.000193
Days within Clutches, linear[d]	1	.004097
Days within Clutches, Quadratic[d]	1	.000001
Eggs within Hens or Remainder (Error B)[e]	741	.000402

[a]The same general model was used in testing all parameters.

[b]Variance associated with determining the experimental mean.

[c]Error for testing effects of pesticides.

[d]Not a source of variation in eggs collected at intervals of 0, 2, 4, 6, 8, 10 and 12 weeks.

[e]Error for testing effects of time and time X diet interaction, and for changes within clutches.

TABLE 2

BODY WEIGHT AND EGG PRODUCTION AND SOME CHARACTERISTICS OF EGGS AND EGGSHELLS OF HENS FED DIELDRIN OR p,p'-DDT

Parameter	Control	Dieldrin 10 PPM	Dieldrin 20 PPM	p,p'-DDT 100 PPM	p,p'-DDT 200 PPM	Experimental MU ± S.E.[a]
Avg. hen wt., g.	1588	1519	1541	1523	1569	1549 ± 16
Avg. No. of eggs collected per bird	69	65	62	68	66	66 ± 3
Avg. wt. of eggs[b], g.	53.1	54.2	55.6	52.9	54.2	54.0 ± 1.1
Avg. No. of eggs in clutch[b]	3.6	3.6	3.4	3.7	3.5	3.5 ± .08
Avg. wt. of dried shell[c], g.	4.9	5.1	5.1	4.9	4.9	5.0 ± .01
Avg. wt. of dried egg contents[c], g.	11.2	11.1	11.5	11.0	11.2	11.2 ± .03
Avg. thickness of shell[c], mm. X 10^{-2}	33.6	33.6	33.7	33.0	33.1	33.3 ± .08
Avg. calcium in shell[c], %	37.2	37.1	36.8	37.1	37.0	36.9 ± .11
Total No. of eggs that could not be collected[d]	2	9	1	4	5	

[a] Experimental mean and its standard error obtained from least squares analysis of variance.
[b] Includes all eggs collected.
[c] Includes eggs collected at the time periods and clutches mentioned in "methods".
[d] Includes eggs with no shells and broken, soft-shelled eggs.

the experiment and was not affected by dietary dieldrin or DDT. On six occasions, three hens refused to consume the full 90 g of feed per day. One of these hens was a control (two occasions) and two were fed 200 ppm DDT. One hen fed 20 ppm dieldrin died from a hemorrhaging cyst on her breast and one fed 10 ppm dieldrin was killed because of a broken leg.

Egg production for the experiment averaged nearly 80% and was not significantly affected by the kind or level of pesticide fed. Although egg production varied among the weekly and 12-day periods, production among these periods was not significantly affected by the insecticides.

Neither level nor kind of insecticide significantly affected the weight of the eggs or the number of eggs per clutch. The eggs of all hens became heavier, as expected, as the experiment progressed. The average number of eggs per clutch would be comparatively less than actual, because both the first and last clutches were interrupted by the discrete beginning and ending of the experiment. Also, the number of clutches laid during the experiment was not affected by feeding insecticides.

Approximately 4,000 eggs were laid during the experiment, of which 1,300 were selected for measuring thickness, weight and calcium in the shell, and for measuring weight of the contents. Considering <u>all eggs</u> measured, there were no significant differences in any of these parameters due to feeding the insecticides. The percent calcium in eggshells was especially constant within treatment groups and among individual hens throughout the experiment. However, thickness and weight of eggshells, and weight of egg contents varied with time.

Twenty-one eggs, randomly distributed across treatments, escaped collection because of broken soft shells or because of the absence of a shell. Nine of these were laid on days in which a normal egg was also collected from the same hen.

Data collected at <u>biweekly</u> intervals for thickness and weight of eggshell and for weight of dried contents are plotted in Figure 1. Mean thicknesses of eggshells for these time periods were .339, .360, .349, .343 and .341 mm for eggs collected from the control hens or from hens fed diets containing 10 ppm dieldrin, 20 ppm dieldrin, 100 ppm *p,p'*-DDT or 200 ppm *p,p'*-DDT, respectively. Mean wieghts of dried eggshell for the corresponding diets were 4.9, 5.3, 5.2, 4.9, and 4.9 g, respectively. These data show that hens fed dieldrin laid eggs with thicker and heavier shells ($P<.05$) than did controls or those fed DDT. DDT did not significantly affect these parameters. Weight of the dried egg contents was not significantly affected by either dieldrin or DDT. Mean thickness or weight of eggshells did not change significantly with time over the 12-week period, while weight of the egg contents increased ($P<.01$) with time. There were no significant insecticide X time

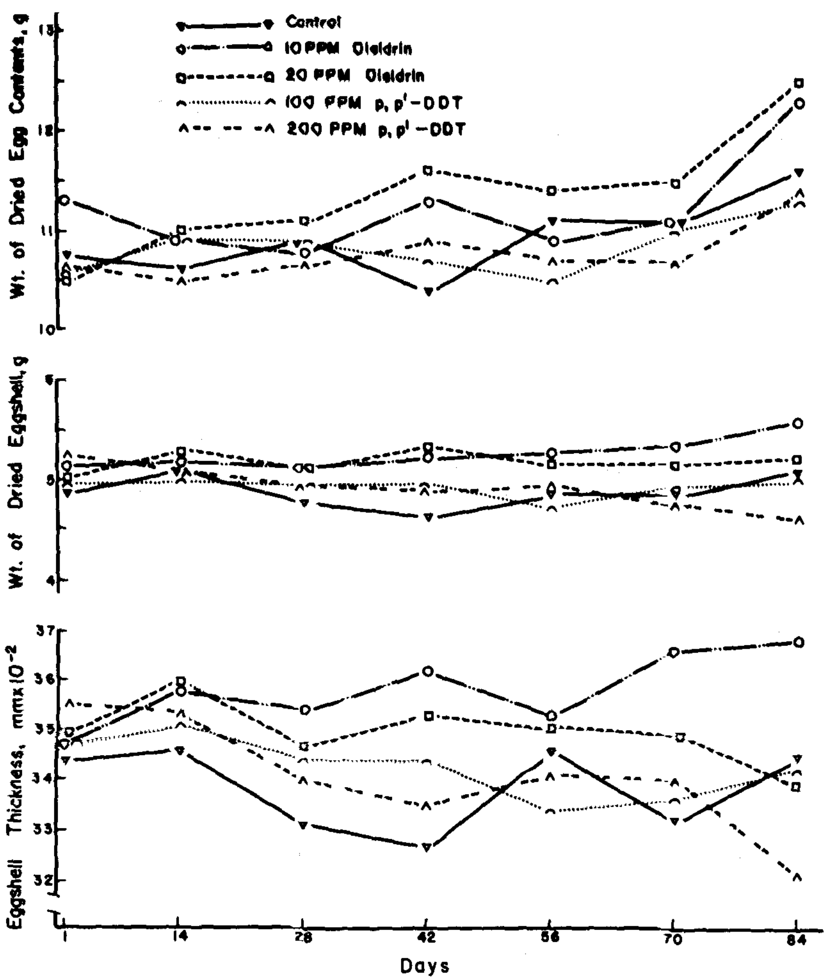

Figure 1. Weight of dried egg contents and eggshell, and shell thickness of eggs collected at biweekly intervals.

interactions in any of the parameters measured.

Thickness and weight of eggshells and weight of dried contents for eggs collected from the specified clutches are shown in Figure 2. In these data, neither insecticides nor time affected eggshell thickness or shell weight, and insecticides did not affect weight of dried contents. However, weight of dried contents increased with time ($P<.01$). Again, there were no insecticide X time interactions.

Within clutches, thickness and weight of eggshells diminished ($P<.001$) by -.0011 mm and -.023 g per day, respectively. Percent calcium in eggshells and weight of dried egg contents were unaffected by position within clutches.

Discussion

Quite high levels of insecticides were fed in these experiments to exaggerate possible effects on egg production or eggshell thickness should such effects occur. In other experiments (16), we have observed that 20 ppm dieldrin in the diet was enough to cause death of some hens. However, no deaths attributed to dieldrin occurred in this experiment. Higher levels of DDT than were fed here can be consumed by chickens or quail without causing death (17,18).

Feed consumption was controlled to standardize consumption of insecticides and nutrients. Bitman (12) fed a low calcium (.56%) diet to Japanese quail in an experiment with o,p'-DDT and p,p'-DDT, where he observed a thinning of eggshells. Others have not specified the calcium level of their diets (5,6,7,9,11) nor have they reported feed consumption. Dietary calcium, phosphorus and vitamin D are known to influence thickness of eggshells (19), but calcium probably would be the nutrient likely to be limiting in most diets.

We found no indication that either p,p'-DDT or dieldrin affected the weight of the eggs, the eggshells, or the dried contents. Normally, the weights of eggs and contents increase with age in young laying hens (19), and this was observed in our experiment. However, Jeffries (20) reported that feeding p,p'-DDT to Bengalese finches reduced egg weight. The change in egg weight he observed averaged 4.3% for birds consuming 0 to 300 μg of DDT per day, but averaged 10% for birds fed 50 to 100 μg of DDT per day. Bitman (12) and Peakall (21) reported similar decreases in egg weight of Japanese quail and ring doves fed p,p'-DDT. Feed consumption data were not given in these reports. Egg size may be reduced in chickens by reducing dietary protein (19). Also, a reduced protein or energy intake may delay egg production in chickens (19) with an apparent decrease in egg size because the first eggs laid are usually smaller than subsequent eggs.

A conflict occurred in our data on eggshell thickness where dieldrin appeared to have increased thickness in eggs collected at biweekly intervals, yet it did not increase thickness in eggs collected in clutches. We suspect that the statistical significance which appeared in the data collected biweekly was due to chance for two reasons: first, eggs from control hens contained thinner shells than eggs from hens fed 10 or 20 ppm dieldrin and eggs from hens fed 10 ppm dieldrin contained thicker shells than eggs from hens fed 20 ppm dieldrin, thus the data did not follow a dose response relationship; second, the analysis for eggs collected in clutches, which also included most of the eggs collected biweekly, did not reveal significant differences among treatments.

These data appear to conflict with results from experiments with ducks (5,8,9), American kestrels (6), sparrow hawks (11) and chickens (10), in which DDT or dieldrin caused thinning of egg-

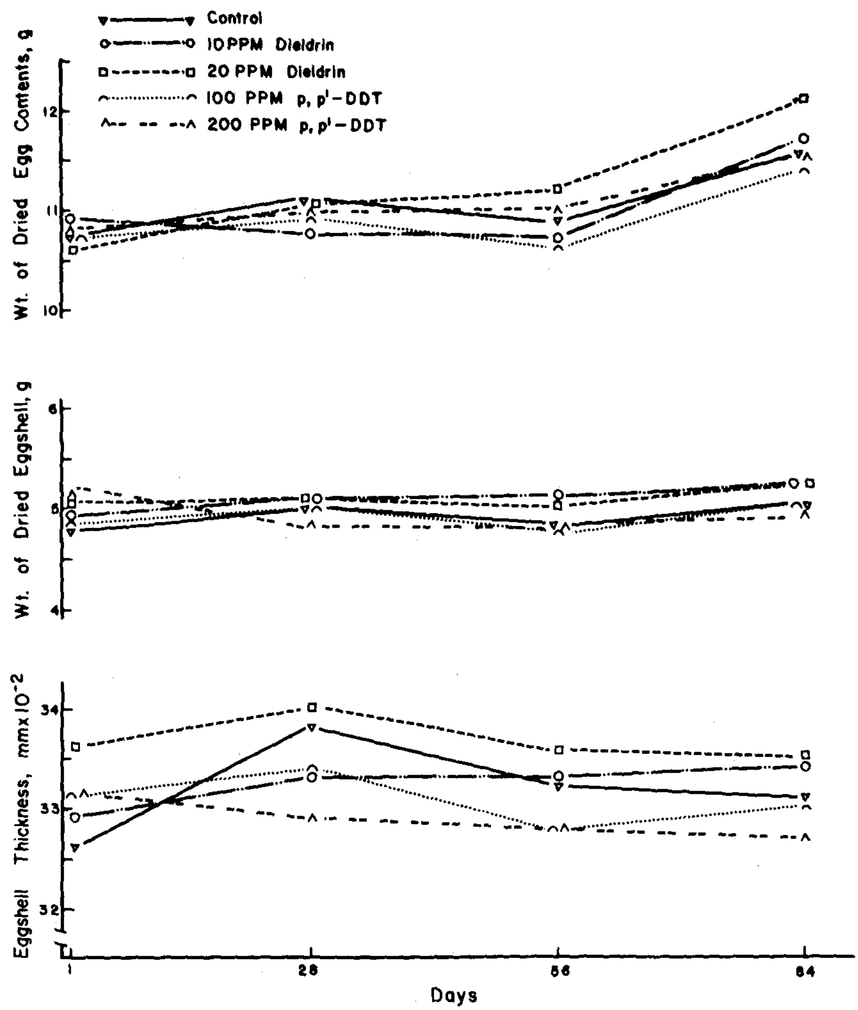

Figure 2. Weight of dried egg contents and eggshell, and shell thickness of all eggs collected in clutches starting nearest days 1, 28, 56 and 84.

shells. A possible explanation for this difference in results could be that some species of birds are more sensitive to these insecticides than others.

Another possible explanation for this apparent conflict could be the statistical treatment of the data. It is known that individual chickens tend to lay eggs within a narrow range of shell thickness, and that this thickness may be different from that of other hens of the same breeding within the flock (19). Because of this identity associated with individual hens, and because the hens were fed and managed individually, the statistical error for testing effects of diets was derived from hens within diets, as shown in Table 1. To have pooled the error associated with eggs within hens would have caused the effects of diets to appear significant. This significance occurs because the error mean square becomes smaller due to the smaller variation in thickness of shells of eggs within hens as opposed to eggs between hens, and because the degrees of freedom for testing the F ratios becomes larger. In cases where hens or birds are kept in pens rather than individually, the pen of birds becomes the experimental unit rather than the individual bird; and, in our model, pens of hens within diets would replace hens within diets.

In a rigorously controlled experiment we conclude that dieldrin or p,p'-DDT, fed at levels up to 20 or 200 ppm, respectively, did not cause thinning of eggshells in chickens fed a nutritionally balanced diet.

Summary

Sixty Leghorn hens were randomly divided into 5 groups of 12 and individually fed a control diet, or diets containing 10 and 20 ppm dieldrin, or diets containing 100 and 200 ppm p,p'-DDT for a 12-week period. Average egg production per bird, egg weight, dry shell weight, shell thickness (not including membranes), and shell calcium were not affected by feeding the insecticides. In addition, the length of the clutch or the number of clutches during the 12-week period were not affected. However, egg weight increased with time during the experiment, and eggshell thickness decreased with successive eggs within a clutch.

Acknowledgments

We thank Gary Richardson, Biometrical Services, Fort Collins, Colorado for statistical processing, Alice Nolin, Patrick Riley, Nancy Jensen and Claudia Creech for technical help, and Daniel Cottrell, Maurice Bush and Kenneth Mize for care of the hens.

References

1. RATCLIFFE, D. A., Nature 215,208 (1967).

2. HICKEY, J. J., and ANDERSON, D. W., Science 162,271 (1968).

3. PEAKALL, D. B., Scientific American 222(4),72 (1970).

4. ANDERSON, D. W., and HICKEY, J. J., The Wilson Bul. 82,14 (1970).

5. HEATH, R. G., SPANN, J. W., and KREITZER, J. F., Nature 224, 47 (1969).

6. WIEMEYER, S. N., and PORTER, R. D., Nature 227,737 (1970).

7. ENDERSON, J. H., and BERGER, D. D., Bio-Science 20,355 (1970).

8. TUCKER, R. K., and HAEGLE, H. A., Bul. Environ. Contam. Toxicol. 5,191 (1970).

9. LEHNER, P. N., and EGBERT, A., Nature 24,1218 (1969).

10. SMITH, S. E., WEBER, C. W., and REID, B. L., Poultry Sci. 49, 233 (1970).

11. PORTER, R. D., and WIEMEYER, S., Science 165,199 (1969).

12. BITMAN, J., CECIL, H. C., HARRIS, S. J., and FRIES, G. F., Nature 224,44 (1969).

13. DAVISON, K. L., J. Agr. Food Chem. 18,1156 (1970).

14. HURWITZ, S., and GRIMINGER, P., J. Agr. Sci. 54,373 (1960).

15. HURWITZ, S., Personal communication (1969).

16. DAVISON, K. L., SELL, J. L., and ROSE, R. J., Bul. Environ. Contam. Toxicol. 5,493 (1970).

17. SMITH, S. I., WEBER, C. W., and REID, B. L., Poultry Sci. 48,1000 (1969).

18. RUBIN, M., BIRD, H. R., GREEN, N., and CARTER, R. H., Poultry Sci. 26,410 (1947).

19. ROMANOFF, A. L., and ROMANOFF, A. J., The avian egg (1949), John Wiley and Sons, Inc., New York.

20. JEFFRIES, D. J., Nature 222,578 (1969).

21. PEAKALL, D. B., Science 168,592 (1969).