Bull. Environm. Contam. Toxicol. 19, 648-654 (1978)

# Influence of Hen Dietary Calcium and Phosphorus on the Integrity of the Egg Shell as It Would Influence Hatching Success and the Consequences of Preincubation 2,4,5-T Spraying with and without a High TCDD Level

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The phenoxyacetic acid herbicides 2,4-D and 2,4,5-T have for many years been the primary chemical combatants against undesirable vegetation. However, disclosure that both compounds were embryotoxic and teratogenic for game birds (LUTZ-OSTERTAG and LUTZ, 1970) and rodents (COURTNEY et al., 1970) generated concern over their continued usage.

Likelihood of adverse effects by 2,4-D and 2,4,5-T on avian reproduction in practical circumstances has since been shown to be negligible (GROLLEAU et al., 1974; HILBIG et al., 1976 a,b; GYRD-HANSEN and DALGAARD-MIKKELSEN, 1974; and SOMERS et al., 1974, 1977). One reason for these results is that the shell is a very effective barrier to herbicide access to the germ. It is common knowledge that shell strength and thickness is quite responsive to maternal dietary inadequacies of calcium. Eggs having weak shells may be more susceptible to herbicide entry in turn affecting hatching success.

At elevated levels, 2,4,5-T is clearly toxic to the embryo (STRANGE et al.,1976); however, the significance of its TCDD contaminant under practical conditions remains obscure. Although the concentration of TCDD through better production techniques is usually less than 0.5 ppm, levels approximating 2 ppm are not out of the realm of likelihood EDMUNDS et al., 1973).

The present study was designed to ascertain the effect of weakening egg shells by altering hen calcium and phosphorus nutrition on incubation performance and the consequences of spraying 2,4,5-T having practical extremes in dioxin content.

0007-4861/78/0019-0648 $01.40

## Materials and Methods

Laying rations were altered to effect marginal inadequacies of calcium and phosphorus (Table 1). Otherwise all resultant diets were isocaloric (2.87 kcal metabolizable energy/g) and isonitrogenous (17% protein). All rations were fed ad libitum to a commercial strain of Single Comb White Leghorn hens over a period corresponding to 36-40 weeks of age. Birds were artificially inseminated with pooled semen from a like breed of cockerels for the last 3 weeks with eggs derived from the final 12 days production being used for spraying and incubation. All data for hen performance and egg measurements fall within these 12 days.

TABLE 1
Composition of Experimental Laying Hen Rations

| Ingredient | % Composition | | | |
|---|---|---|---|---|
| | Normal | Low Ca | Low P | Low Ca & P |
| Constant | | | | |
| Corn | | | 66.45 | |
| Soybean meal (49%) | | | 23.00 | |
| Tallow | | | 1.00 | |
| Salt | | | 0.25 | |
| Vit-Min. Mixes[a] | | | 0.75 | |
| DL-Methionine | | | 0.05 | |
| Variable | | | | |
| Limestone | 6.50 | 2.65 | 6.90 | 3.08 |
| Dicalcium Phosphate | 2.00 | 2.00 | 1.25 | 1.25 |
| Kaolin | - | 3.85 | 0.35 | 4.17 |
| Calculated Analysis | | | | |
| Calcium, % | 2.96 | 1.50 | 2.96 | 1.50 |
| Total Phosphorus,% | 0.57 | 0.57 | 0.42 | 0.42 |

[a]The vitamin and mineral premixes contributed the following per kg of complete feed: vit. A 8000 I.U.; vit. $D_3$ 1600 I.C.U.; vit. E 11 I.U.; vit. $B_2$ 7 mg; pantothenic acid 7 mg; vit. $B_{12}$ 9 mcg; niacin 20 mg; choline 900 mg; vit. K 1.5 mg; folic acid 1.5 mg; biotin 0.25 mg; ethoxyquin 125 mg; Mn 55 mg; Zn 50 mg; Cu 5 mg; Fe 30 mg.

All eggs to be incubated were sprayed with a volume equivalent to 746 l/ha. Control eggs were treated with water while 2,4,5-T application approximated 10x normal usage rate (11.2 kg/ha). There were two treatments involving 2,4,5-T. The first involved a commercial formulation known to contain less than 0.1 ppm TCDD. The second mix[1] used the same lot 2,4,5-T mix only 2 ppm of TCDD was added.

[1]Trade name Esteron 245 (Lot #MM 675413) through the courtesy of Dow Chemical Co., Midland, Michigan.

After spraying, eggs were allowed to air dry prior to incubation. On day 18, eggs were candled and all viable eggs were transferred to a hatcher. The conditions and equipment surrounding spraying and incubation are the same as described earlier by SOMERS et al. (1974). Upon hatching late and pipped dead germs, as well as chicks, were examined for gross morphological aberations. Four replicate groups of chicks from each sex represented the preceeding hen dietary and egg spray treatments in a subsequent 4 week growing period. All birds were maintained in electrically heated raised floor brooder batteries and received water and a commercial chick starting ration ad libitum.

Samples of egg contents, shells, late dead germs, and chicks were saved for 2,4,5-T residue analysis. Clean-up methodology followed that of YIP (1971) as modified by SIRONS (1974). Gas-liquid chromatography parameters were as previously described by SOMERS et al. (1974). TCDD content of tissues was not quantified.

## Results and Discussion

Feeding laying hens rations with a lower than requirement level of calcium had repercussions on rate of production and egg shell strength (Table 2). It is interesting to note that there was an increased porosity estimate

TABLE 2

Influence of Dietary Alterations in Calcium and Phosphorus on Hen Performance and Egg Quality[a]

| Ration | | Production | Egg | | |
|---|---|---|---|---|---|
| Ca | P | % H.D.B.[b] | Wt.,g | Porosity[c] | Strength[d] |
| Norm. | Norm. | 71.1[y] | 60.5 | 1.36[y] | 23.9[y] |
| Low | Norm. | 59.5[z] | 60.0 | 1.84[z] | 35.8[z] |
| Norm. | Low | 72.4[y] | 60.6 | 1.31[y] | 23.0[y] |
| Low | Low | 54.5[z] | 58.9 | 1.48[y] | 35.4[z] |
| σ(140) df) | | 6.7 | 2.4 | 0.35 | 8.9 |

[a]The data in any one column with superscripted letters refers to Duncan's Multiple Range Test. Figures without a common letter are significantly different ($P<0.05$).

[b]Hen production on a hen-day basis.

[c]Porosity is expressed as the percentage egg weight evaporative loss over 10 days of storage at 15.5°C and 80% relative humidity.

[d]Shell strength is expressed in μ deformation from normal shape imposed by a 500 g weight at the equator. The greater the value the weaker the shell.

when calcium alone was low but not when it was accompanied by a reduced phosphorus level. There were no indications that lowering dietary phosphorus had any impact on hen performance; however, this result was expected with the present submarginal inadequacy. According to HARMS et al. (1977) this type of deficiency is more likely to cause the syndrome "cage layer fatigue" and mortality than affect performance. The data support that with a lower dietary phosphorus and/or calcium in the present experiment, there resulted readily measurable changes in egg shell quality.

When these eggs were subsequently contaminated with 2,4,5-T or the formulation containing added TCCD and then incubated, data on all parameters assessing embryological development failed to indicate that there was any statistically significant interaction with hen dietary stress ($P>0.05$). Thus, eggs having shells weakened by inadequate maternal macro-mineral nutrition were not any more subject to a 10x normal dose of 2,4,5-T or the high TCDD formulation than those originating where this dietary aspect was normal.

TABLE 3

Incubation Performance of Hens' Eggs[a]

| Treatments | | NVG | % of VG | | | | Terata |
|---|---|---|---|---|---|---|---|
| | | % Total[b] | EDG | LDG | PDG | Hatch | %VG-EDG |
| Level | | | | | | | |
| Ca | P | | | | | | |
| Norm. | Norm. | 3.5[y] | 7.1 | 3.1 | 1.0 | 88.7 | 0.7 |
| Low | Norm. | 5.3[y] | 11.1 | 4.1 | 0.0 | 85.2 | 2.8 |
| Norm. | Low | 4.0[y] | 7.4 | 2.1 | 0.5 | 90.3 | 0.6 |
| Low | Low | 11.8[z] | 5.6 | 2.4 | 0.9 | 89.4 | 1.5 |
| Effect of 2,4,5-T & 2,4,5-T:TCDD Spraying | | | | | | | |
| Treatments | | | | | | | |
| Water Control | | 6.0 | 7.6 | 2.6 | 1.1 | 88.8 | 1.6 |
| 2,4-5-T | | 5.7 | 9.6 | 3.1 | 0.3 | 87.1 | 1.0 |
| 2,4,5-T:TCDD | | 6.7 | 7.5 | 3.1 | 0.7 | 89.3 | 1.4 |
| σ(66 df) | | 4.4 | 5.9 | 2.2 | 0.5 | 6.5 | 0.6 |

[a]Means based on 432 eggs over 12 replicates/ration/spray treatment. NVG=non-viable germs; VG=viable germs; EDG=early dead germs (deaths prior to 6 days incubation); LDG= late dead germs (deaths after 6 days); PDG=pipped dead germs (deaths after breaking shell but not successful in hatching.

[b]See footnote a, Table 2.

An orthogonal partitioning of means by main effects for data associated with incubation performance is shown on Table 3.

The only statistically significant alteration detected was with respect to maternal diet and non-viable germs. Hens which were fed a ration deficient in both calcium and phosphorus had a greater proportion of germs which failed to develop than occurred with any other dietary treatment; however, the proportion of viable germs leading to normal chicks was unaffected. Eggs sprayed with 2,4,5-T or 2,4,5-T having TCDD added in excess of 10x normal were comparable in their development to the water treated controls in every parameter. Terata encountered were not different regardless of experimental variable and in proportions as well as appearance to that encountered in normal hatchery operation.

Analyses for 2,4,5-T residues within the egg after spraying and associated with the EDG, LDG and chick all indicate there were no ready differences in concentration which could have evolved because of hen nutrition (Table 4). Hence, even though weaker shells were shown to exist they were still an effective barrier to herbicide entry. The observations that the 2,4,5-T residue once within the egg subsequently decreased with incubation and hatching of the chick agrees with the earlier studies of GROLLEAU *et al.* (1974) and SOMERS *et al.* (1974) as do their absolute concentrations.

TABLE 4

Effect of Hen Dietary Calcium and Phosphorus on the 2,4,5-T Levels in the Spray Contaminated Egg Before and After Incubation[a]

| Ration | | Concentration (ppm±std. dev., wet weight) | | | |
|---|---|---|---|---|---|
| Ca | P | Egg[b] | EDG | LDG | Chick |
| Norm. | Norm. | 0.30(0.07) | 0.48(0.18) | 0.33(0.06) | 0.23(0.07) |
| Low | Norm. | 0.46(0.19) | 0.38(0.02) | 0.32(0.05) | 0.28(0.13) |
| Norm. | Low | 0.28(0.07) | 0.53(0.14) | 0.46(0.06) | 0.24(0.11) |
| Low | Low | 0.36(0.18) | 0.49(0.16) | 0.31(0.02) | 0.26(0.12) |
| Total Tissue $\bar{x}$ | | 0.35(0.18) | 0.47(0.17) | 0.36(0.08) | 0.24(0.11) |

[a] Pre-incubation 2,4,5-T residues from shells and membranes across rations averaged 65.61 ± 5.89 ppm.

[b] Content within the egg *ca.* 24 hours after contamination and before incubation.

The data associated with chick growth to 4 weeks of age are not shown. A statistical analysis of weight gain and mortality occurring during that period failed to reveal any evidence of an effect due to either the main variables of maternal diet and herbicide spray contamination or their interaction regardless of sex. WHITEHEAD and PETTIGREW (1972) observed that 21 days ingestion of a ration containing 100 mg 2,4,5-T/kg increased weight gain of chicks. Considering the low levels associated with chicks of the

present study and absence of any intentional dietary contamination it is not unexpected that there was neither a positive nor negative effect on early live performance. Overall body weights after 28 days averaged 310 and 266 g for males and females, respectively.

It is well accepted that the dioxin compounds are embryotoxic as well as teratogenic and constitute a serious environmental threat. According to EDMUNDS et al. (1973) who analyzed several commercial 2,4,5-T sources, the occurrence of TCDD is normally less than 0.5 ppm and seldom exceeds 2 ppm. The present study employed 2,4,5-T further contaminated with 2 ppm as a practical extreme, then used this mix at 10x recommended rate. In every instance there was no indication of an adverse effect on either embryo development or early performance of subsequent chicks. In general, the many reports documenting problems with TCDD all greatly exceeded this feasible concentration extreme and/or resorted to unlikely routes of administration.

## Summary

Commercial strain laying hens were fed practical rations deficient in calcium and/or phosphorus to affect weak shelled eggs. Spraying these eggs prior to incubation with 2,4,5-T preparations having extremes in TCDD contamination at 10x recommendation was without effect on all parameters used to evaluate embryological development and subsequent early performance of resultant chicks. Analyses for 2, 4,5-T residues indicated that the shell though weakened was unaffected as a barrier to herbicide contamination.

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